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CORRELATION STUDIES OF EGG PRODUCTION AND POSSIBLE GENETIC INTERPRETATIONS[1]

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INTRODUCTION

Many investigators have published results dealing with the correlation between each of several different variables and egg production. Their investigations have dealt with the effects, on egg production, of environmental influences, such as date of hatch and date of first egg, and with the effects of several variables that have been regarded as hereditary. It is important, from the practical as well as from the scientific viewpoint, that investigations of this kind be extended (1) to ascertain accurately the influence of the date of hatch and the date of first egg upon egg production during the first year; (2) to find adequate criteria for predicting or estimating relative egg production; and (3) if possible, to establish any linkages that may exist as indicated by the correlation coefficients. To obtain information on these points, the investigation reported in this paper was undertaken.

Several reviews of the existing literature on the subject have been published by investigators in this field, particular attention being called to those of Kempster (*19*, *20*, *21*),[2] Hays and Sanborn (*12*), and Knox (*24*, *25*, *26*).

EXPERIMENTAL PROCEDURE

In this experiment, 903 Rhode Island Red pullets and 884 Single-Comb White Leghorn pullets were used in 1928, 1929, and 1930. These birds were markedly free from disease, and only a very small percentage of the original number died during the course of the experiment. There was no selection of the pullets, and each bird that survived was kept in the laying house for 365 days from the date of first egg. All the birds were fed the same diet, housed similarly each year, and otherwise kept under as nearly the same conditions as possible in order to make the data strictly comparable. The work was carried on at the United States Animal Husbandry Experiment Station, Beltsville, Md.

The factors which were considered environmental, date of hatch and date of first egg, were studied to determine whether or not they had any influence on production and also what correlation, if any,

[1] Received for publication Jan. 9, 1935; issued June 1935.

[2] Reference is made by number (italic) to Literature Cited, p. 583.

they might have with possible hereditary characters. The date of hatch of the Rhode Island Reds was approximately from the middle of March to the middle of May each year, the birds being hatched in eight weekly periods. The date of hatch of the White Leghorns was from the last week in March to the middle of May, in seven weekly periods.

The possible hereditary characters considered were: Sexual maturity (age at first egg), number of eggs laid during the first 50 days after sexual maturity, number of eggs laid before March 1, percentage of production before March 1, length of winter pause, number of eggs laid during the last 50 days of the 365-day period, and number of eggs laid during August and September of the year following the date of hatch. These characters were studied in both the White Leghorns and Rhode Island Reds. One other hereditary character, broodiness, was used for Rhode Island Reds only, because practically no broodiness existed in the strain of White Leghorns. Therefore, this factor is discussed separately near the end of the paper.

Most of the foregoing characters have been studied in previous investigations. However, since some of them seem to be measures of the same hereditary characters, this study deals with the data pertaining to them in various ways in order to determine, by the use of corrected partial and multiple correlation coefficients, which of the factors may be significantly correlated with total egg production but independent of the environmental influences considered and also independent of other hereditary characters.

With respect to the partial correlation, Wallace and Snedecor (*30, p. 56*) state:

> To the novice, a word of warning should be said concerning a phrase which has become popular in biological literature. We read of $r_{DE.ABCX}$ as the partial correlation between D and E, "the other variables being held constant." As a matter of fact, $r_{DE.ABCX}$ is an average of a number of simple correlations, r_{DE}, in groups having various values of A, B, C and D. There is no necessity of postulating the constancy of these variables. The very genius of the partial correlation coefficient is that it gives an average measure of association between two variables *independent* of the accompanying variation of the other variables. The persons using this phrase are limiting their thinking to an ideal situation in which experimental control is substituted for statistical control. This seems to us to be an unfortunately restricted attitude to take toward partial correlation.

In the interpretation of the partial correlation coefficient, it is decidedly worth while to take cognizance of this criticism by Wallace and Snedecor. All tests for the significance of simple, partial, and multiple correlations and t values are taken from the table for significant values for r, R, and t by Wallace and Snedecor (*30*).

RESULTS AND DISCUSSION

Table 1 contains all the simple correlation coefficients between the dependent variable, total egg production, and each of nine independent variables. Table 2 gives the means and standard deviations for the different variables. The standard deviation is useful as a measure of absolute variation in comparing the variability of one breed with that of another with respect to the same character, or of one character with another in the same breed or different breeds.

TABLE 1.—*Simple correlations between various egg-production factors and between each factor and total production in White Leghorns and Rhode Island Reds* [1]

Egg-production factor	Breed	Simple correlation between factors indicated								
		Date of first egg	Sexual maturity	Eggs first 50 days	Eggs to Mar. 1	Length of winter pause	Percentage of egg production to Mar. 1	Eggs August and September	Eggs last 50 days	Total production
Date of hatch	White Leghorns	+0.605	+0.220	−0.040	−0.364	−0.119	+0.036	−0.024	−0.291	−0.158
	Rhode Island Reds	+0.427	+.018	−.031	−.206	−.140	+.090	+.078	−.212	+.068
Date of first egg	White Leghorns	.....	+.901	−.033	−.614	−.175	+.033	−.085	−.472	−.288
	Rhode Island Reds.	.....	+.897	−.115	−.615	−.209	+.018	+.022	−.344	−.240
Sexual maturity (age at first egg), days.	White Leghorns	.....	.....	−.022	−.562	−.155	+.022	−.093	−.427	−.272
	Rhode Island Reds.	.....	.....	−.112	−.580	−.171	−.015	−.015	−.340	−.296
Eggs first 50 days, number.	White Leghorns	.....	.....	.....	+.498	−.239	+.619	+.122	+.117	+.403
	Rhode Island Reds.	.....	.....	.....	+.510	−.273	+.654	+.134	+.162	+.477
Eggs to Mar. 1, number.	White Leghorns	.....	.....	.....	.....	−.496	+.727	+.250	+.413	+.680
	Rhode Island Reds.	.....	.....	.....	.....	−.511	+.750	+.110	+.342	+.655
Length of winter pause, days.	White Leghorns	.....	.....	.....	.....	.....	−.774	−.179	−.054	−.422
	Rhode Island Reds.	.....	.....	.....	.....	.....	−.824	−.125	−.041	−.443
Egg production to Mar. 1, percent.	White Leghorns	.....	.....	.....	.....	.....	.....	+.252	+.172	+.626
	Rhode Island Reds.	.....	.....	.....	.....	.....	.....	+.149	+.150	+.622
Eggs August and September, number.	White Leghorns	.....	.....	.....	.....	.....	.....	.....	+.725	+.692
	Rhode Island Reds.	.....	.....	.....	.....	.....	.....	.....	+.675	+.652
Eggs last 50 days, number.	White Leghorns	.....	.....	.....	.....	.....	.....	.....	.....	+.660
	Rhode Island Reds.	.....	.....	.....	.....	.....	.....	.....	.....	+.667

[1] Correlations to be fairly significant must be 0.068 for White Leghorns and 0.062 for Rhode Island Reds; to be highly significant, they must be more than 0.094 for White Leghorns and 0.088 for Rhode Island Reds.

TABLE 2.—*Means and standard deviations for the characters studied*

Character studied	White Leghorns [1]		Rhode Island Reds [2]	
	Means	Standard deviations	Means	Standard deviations
Sexual maturity (age at first egg)....days	180.9	27.6	199.6	29.8
Eggs first 50 days....number	28.2	8.5	31.2	8.9
Eggs to Mar. 1....do	77.6	26.6	74.2	28.0
Percentage of production to Mar. 1	53.6	14.5	56.0	16.2
Length of winter pause....days	20.7	21.3	21.6	23.4
Eggs August and September....number	22.3	14.8	23.6	13.3
Eggs last 50 days....do	16.5	13.4	16.7	12.4
Total egg production....do	191.4	46.9	191.9	44.6

[1] Mean date of hatch, Apr. 10. Mean date of first egg, Oct. 7.
[2] Mean date of hatch, Apr. 4. Mean date of first egg, Oct. 19.

DATE OF HATCH

It is evident from the data in table 1 that in both breeds studied there is a high and significant simple correlation between date of hatch and date of first egg. This was to be expected since it is logical to believe that the earlier a pullet is hatched the earlier she will lay her first egg.

The White Leghorn pullets have a simple correlation coefficient between date of hatch and sexual maturity of +0.220, which, statistically, is highly significant. The Rhode Island Reds, however, have an insignificant correlation of only +0.018 between these two characters. The White Leghorns also have a highly significant simple correlation of −0.158 between date of hatch and total egg production, whereas the Rhode Island Red pullets have only a barely significant coefficient of +0.068 between the same two characters. Therefore, the difference between the two breeds in the correlations between date of hatch and total egg production is probably due to the difference in their sexual maturity. If this assumption is true, the corrected partial correlation coefficient between the date of hatch and total egg production should be insignificant. The other variables included were age at first egg, date of first egg, winter pause, percentage of production, and the number of eggs laid during August and September. The assumption was found to be true, the coefficients being +0.042 for the White Leghorns and −0.054 for the Rhode Island Reds.

Hays, Sanborn, and James (*13*), Kempster (*21*), Upp and Thompson (*29*), and Knox (*26*), obtained more or less significant and sometimes contradictory results. The differences among the data of the various investigators may be ascribed to the reasons that Kempster (*21*) gives in his conclusions: That the date of hatching and its association with sexual maturity is dependent on flock management, and that late hatching tends slightly to reduce the age at which the first egg is laid. In addition, it may be due to the difference in the range of the date of hatch.

Knox (*26*) has shown that there is a curvilinear correlation between the date of hatch and the total number of eggs produced in the pullet year for the 17 weekly hatches which he studied. Hence, different correlations might be expected, depending on the part of the curve from which the data were taken. If the data were taken from the fore part of the curve it would be positive; from the middle part, insignificant; and from the last part, negative. When the hatching period used in the present study is compared with a similar period in Knox's study, there is little evidence in either case of any significant correlation. The hatching period of 8 weeks used in this study is probably the most practical and economical one and the one that is most used by commercial poultrymen.

It is also possible that if the other investigators had used a corrected partial correlation coefficient a much lower or even an insignificant correlation would have been found between the date of hatch and total egg production.

The data of Hays (*8*) are at variance with the data obtained in the present study, that is, that hatching date has no significant effect upon total egg production. He states (*8*, *p. 8*):

> These [coefficients of variation], together with the gross data presented in the table, show clearly that hatching date does affect annual egg production and variability in production.

It is questionable whether the data given by him show anything except normal variation. It will also be noted that as the number of individuals in each group increased materially, the coefficient of variation of the means decreased, a normal expectation. In any event, the coefficient of variation is a relative measure of variation

and shows whether or not there is more variability in one character than in another. Hence, the gross data, and not the coefficients of variation, presented by him showed the effect of hatching date upon annual egg production.

If hatching date affects egg production, the gross data of Hays (*8*) for the 4 years, if compiled in eight weekly hatches, should show a definite trend in egg production. When the data are thus compiled, it is seen that hatching date has little or no effect on egg production. What trend is apparent is directly opposite to that usually reported, which is that the earlier a bird is hatched the more eggs she will lay in her first laying year. However, when the average egg production of the birds from the first 4 hatches is compared with the average egg production of the birds from the last 4 hatches it is found that there is a difference of only 16+ eggs, hardly enough to be significant. Even if the difference were significant it would still be necessary to find out whether or not there would be a significant partial correlation. As has been shown in this investigation, it is possible to obtain a significant correlation coefficient and yet have an insignificant corrected partial correlation coefficient.

DATE OF FIRST EGG

The correlation coefficients of +0.901 and +0.897 for the White Leghorn and Rhode Island Red pullets, respectively, between date of first egg and sexual maturity are very high (table 1). Sexual maturity is obtained by determining the number of days elapsing between the date of hatch and the date of first egg. Hence, if the hatching date were held constant, sexual maturity would be the controlling factor in establishing the date of first egg, in which case the simple correlation coefficient between sexual maturity and date of first egg would be 1.00. Even when the date of hatch varies over an interval of 7 or 8 weeks, as was the case in the present study, a high correlation may be expected between these two variables. The high correlation coefficients between them in this study agree with the data of Jull (*16*), and with the data of Knox (*25*) when the date of hatch was limited to a period of 7 or 8 weeks.

The simple correlation coefficients between date of first egg and total egg production were found to be highly significant in both breeds, −0.288 for the White Leghorns and −0.240 for the Rhode Island Reds. However, as stated by Knox (*24, p. 249*)—

> It would be of considerable value for predicting the future egg production of pullets to know exactly what this association would mean statistically and whether or not the date of first egg is more important than sexual maturity.

He further states (*24, p. 249*):

> * * * it is difficult to differentiate between them, but a multiple correlation study and the use of partial regression coefficients ought to give a true indication of the actual statistical value of each with winter, spring, and annual egg production.

With this in mind, the writers calculated the partial correlation coefficient between the date of first egg and total egg production and found it to be of insignificant value in both breeds, being −0.016 for the White Leghorn and −0.055 for the Rhode Island Red pullets. In obtaining the partial correlation coefficients, the other variables included were date of hatch, sexual maturity, percentage of produc-

tion to March 1, and egg production during August and September of the year following date of hatch.

The data presented concerning the simple correlation coefficients agree with the data of Jull (*16*), Kempster (*20*), Maw and Maw (*27*), Hauschildt (*7*), and Knox (*25*). However, Kempster and Knox each found a curvilinear relationship between date of first egg and total egg production, which was probably due to the fact that their data included a greater range in the date of hatch and date of first egg than is included in this investigation. When the data of the different investigators are compared empirically month for month the same relative association is found as for the period considered in this investigation. However, the partial correlation coefficient, which none of the previous investigators included, shows that the date of first egg is not significantly correlated with total egg production and that the significant simple correlation coefficients found in this study, and probably in the previous investigations mentioned, are due to some other factor, possibly sexual maturity.

Therefore, within the limits of this study, neither of the two environmental factors considered—date of hatch and date of first egg—has any significant correlation with total egg production, as shown by the corrected partial correlation coefficient with total egg production.

SEXUAL MATURITY

Sexual maturity, already defined as the number of days that elapse between the date that the pullet is hatched and the date that she lays her first egg, is the first hereditary character considered in this paper. The same character has been variously designated by other investigators as age in days laying commences, maturity, sexual maturity, and age at first egg.

The simple correlation coefficients, between sexual maturity and total egg production, of -0.272 for the White Leghorns and -0.296 for the Rhode Island Reds are relatively large and highly significant. The partial correlation coefficients are even higher, -0.398 for the White Leghorns and -0.479 for the Rhode Island Reds, the other variables considered being winter pause, percentage of production to March 1, and number of eggs during August and September of the year following the date of hatch. Hence it is apparent that sexual maturity is highly and significantly correlated with total egg production.

Similar observations concerning the simple correlations have been made by Ball and Alder (*1*), Kennard (*22*), Hervey (*14*), Hays and Bennett (*9*), Hays, Sanborn, and James (*13*), Jull (*16*, *17*), Kempster (*19*, *20*), Parkhurst (*28*), Knox (*23*), Buster (*2*, *3*), and Graham.[3] None of these investigators calculated any partial correlation coefficients.

The simple correlation coefficients between sexual maturity and total egg production obtained by Hays and Sanborn and by the present authors are in agreement, but the partial correlation coefficients are not. Hays and Sanborn obtained a partial correlation coefficient of -0.0238 ± 0.0177, which is insignificant. The authors, on the other hand, obtained a significant partial correlation co-

[3] GRAHAM, J. C. THE CORRELATION OF BODY WEIGHT AT FIRST EGG AND AVERAGE MONTHLY AND ANNUAL EG.. WEIGHT IN RHODE ISLAND REDS. (Unpublished manuscript.)

efficient of −0.479 for the same breed. This seeming lack of agreement may be due, however, to the highly significant simple correlation coefficient, −0.5956, which Hays and Sanborn observed between sexual maturity and their particular measure of persistence. Their measure of persistence, which is discussed more fully later, seems questionable because the high correlation between sexual maturity and the measure of persistence that they used was probably responsible for lowering the partial correlation coefficient between sexual maturity and egg production.

RATE OF LAYING

Four different measures of the rate of laying were studied, namely: Number of eggs laid during the first 50 days of production, number of eggs laid to March 1, length of winter pause, and percentage of production to March 1. There may be some question as to whether these four measures of rate are comparable. If they are comparable, then a high correlation coefficient should be obtained between each pair of the four measures and similar correlations should be obtained between each measure and total egg production.

The correlation coefficient between the number of eggs laid the first 50 days and date of hatch is insignificant for both the White Leghorns and the Rhode Island Reds (table 1). The correlations between the former character and each of the two characters, date of first egg and sexual maturity, although insignificant in the case of the White Leghorns, are low but highly significant in the case of the Rhode Island Reds. The correlations between the number of eggs the first 50 days and each of the four characters, eggs to March 1, winter pause, percentage of production to March 1, and total production, are high and significant in both breeds.

Evidently the number of eggs laid the first 50 days is influenced relatively little by the date of hatch and has practically no correlation with date of first egg and sexual maturity, except in the Rhode Island Reds, in which case the correlation coefficient is −0.115. However, it has a high and significant simple correlation with the other measures of rate and with egg production. The number of leggs laid the first 50 days has a higher partial correlation with egg production than the simple correlation coefficient, being +0.461 and +0.518 for the White Leghorns and Rhode Island Reds, respectively. This measure of rate, therefore, is a good one to use, provided no other one has a higher partial correlation coefficient with annual egg production and no significant correlation coefficient with environmental factors or other hereditary factors.

The number of eggs laid to March 1 has been considered by several investigators as winter egg production. Table 1 shows that the number of eggs laid to March 1 is highly and significantly correlated with the date of hatch and the date of first egg in both breeds. It is also highly correlated with the other measures of rate and with total egg production. The partial correlation between the number of eggs to March 1 and total egg production was found to be +0.605 for the White Leghorns and +0.735 for the Rhode Island Reds.

Because this measure of rate is highly correlated with the two environmental factors studied, it is not so desirable a measure to use as is the number of eggs laid in the first 50 days. This seems to be

obvious even though the number of eggs laid to March 1 has a higher correlation with the other measures of rate and with egg production.

The number of eggs laid before March 1 has been used as a measure of rate by many investigators in genetic and statistical studies of egg production. When the previously mentioned facts regarding this factor are considered, it is evident that any adequate genetic or statistical interpretation is not only difficult but hazardous and in all probability should not be used in a study of egg production. This, of course, does not preclude the importance of economic studies of winter egg production.

Winter pause, another measure of rate studied, has been considered variously by different investigators. In this investigation, winter pause is considered as the accumulated number of days that production has ceased for periods of 4 days or more.

The data in table 1 show that winter pause, as defined, is significantly correlated with the date of hatch, date of first egg, and sexual maturity. Any one of these correlations would be sufficient to eliminate it from consideration, even though it is highly correlated with the other measures of rate and with total egg production. This is substantiated by the partial correlation coefficients of +0.049 for the White Leghorns and −0.077 for the Rhode Island Reds between winter pause and total egg production, when percentage of production to March 1 is used as one of the other variables. These coefficients show that winter pause has very little actual correlation with total egg production. Therefore, such a measure is not only significantly correlated with the environmental factors studied but as a reciprocal is more or less a statistical duplication of the percentage of production to March 1.

In addition to this, winter pause is difficult to use in studies on egg production. In this connection, Goodale and Sanborn (*5, p. 115*) made the following observation:

> The trait [winter pause] does not lend itself to ordinary statistical treatment because of its nature, which depends partly on an inherent condition of the strain, and partly on environmental conditions, particularly those that determine the time of year when birds begin to lay. Further, it is possible that more than one cycle is involved.

Then, again, Jull *(18, p. 226, 227)*, in his study of factors affecting egg production, states that—

> No attempt has been made to separate the pauses occurring in each bird's record according to whether they were caused by external factors or were due to inheritance, because no method has been made known whereby the facts can be determined.

Therefore, because of the difficulty of determining a satisfactory measure of winter pause, the inability to separate the influence of environment and inheritance, its doubtful value as shown by the present correlation study, and its insignificant partial correlation with egg production, winter pause should be eliminated from consideration in any attempt to solve the problem of the inheritance of egg production.

The last measure of rate used is the percentage of egg production from the date of first egg to March 1. This measure has been termed "intensity of production" by some writers. Hurst (*15*) was probably the first investigator to study this particular measure of rate. Few

investigators have seen fit to use it, yet the data in table 1 show that it is ideal. It has practically no correlation with the date of hatch for the White Leghorns, and a coefficient of only +0.090 for the Rhode Island Reds and practically no correlation with date of first egg and sexual maturity for either breed. In addition, it has a high simple correlation coefficient with total egg production (+0.626 for the White Leghorns and +0.622 for the Rhode Island Reds) and a correspondingly high partial correlation coefficient (+0.555 for the White Leghorns and +0.568 for the Rhode Island Reds).

The data agree with those of Hays and Sanborn (*11*), who obtained a correlation coefficient of $+0.48 \pm 0.01$ between rate of production (percentage of production to March 1) and total annual production for 2,528 Rhode Island Red pullets. Knox (*23*) obtained a correlation coefficient of $+0.34 \pm 0.08$ between rate of production based on the 2 consecutive months of highest production (another measure of rate) and total annual production in White Plymouth Rocks.

However, the data of Hays and Sanborn (*11*) showed a significant correlation in Rhode Island Reds between rate of production and sexual maturity, the coefficient being -0.23 ± 0.01, whereas the present authors found a correlation coefficient of +0.022 in the White Leghorns and of −0.015 in the Rhode Island Red pullets, respectively, signifying practically no correlation. It may be that the lack of agreement is due to the different methods of calculating rates. Hays and Sanborn (*11*) calculated rate, as reported by them (*10*), by using the number of eggs laid from the date of first egg to March 1, divided by the number of days from first egg to March 1, less all pauses of 4 or more days from November 1 to March 1. The authors figured percentage of production to March 1 in a similar manner but included the pauses, a method which seems less arbitrary than the one used by Hays and Sanborn (*11*).

From these data it is apparent that percentage of egg production from date of first egg to March 1 should be used to a greater extent in future statistical and genetic investigations of egg production than has been done in the past. It is much to be preferred to the more generally used measure of rate, the number of eggs to March 1, or to winter pause. It is also a better measure of rate than the number of eggs laid in the first 50 days of production as measured by the size of the correlation coefficients with egg production and lack of correlation with the environmental factors used.

PERSISTENCE

The two measures of persistence chosen were the number of eggs laid during the last 50 days of the 365-day period and the number of eggs laid during August and September of the year following the date of hatch of the pullets. These were studied in the same manner as the different measures of rate.

As shown by table 1, the number of eggs laid during the last 50 days of the laying year is evidently highly and significantly correlated with the environmental factors considered and with sexual maturity and percentage of production to March 1, although least with respect to the latter. It also has a very high and significant simple correlation coefficient with total egg production, being +0.660 and +0.667 for the White Leghorns and Rhode Island Reds, respectively. The

partial correlation coefficients were slightly higher in both breeds, being +0.667 for the White Leghorns and +0.700 for the Rhode Island Reds. In spite of these high correlations with total egg production, the number of eggs laid the last 50 days should be eliminated from all statistical and genetic studies of egg production because of the high correlations between this factor and each of the following: Date of hatch, date of first egg, sexual maturity, and percentage of production before March 1.

The simple correlation coefficients of the number of eggs laid in the last 50 days with date of hatch, sexual maturity, percentage of production to March 1, and total egg production, agree substantially with the results reported by Hays and Sanborn (*10, 12*). The same is true of the partial correlation coefficients. This would indicate that the number of eggs laid the last 50 days of the 365-day period, used by the authors, and the length of the laying period tabulated in class intervals of 15 days, the method used by Hays and Sanborn (*10*), are similar measures of persistence. Harris and Lewis (*6*) obtained a correlation coefficient of -0.2438 ± 0.0302 between date of first egg and date of cessation of laying, which agrees substantially with the correlation coefficient given in table 1 between date of first egg and number of eggs laid the last 50 days of the laying year. The reasons given by the authors for eliminating from consideration the number of eggs laid in the last 50 days of the laying year are no doubt applicable to the length of the laying period, and probably to the date of cessation of laying as reported by Hays and Sanborn (*10*) and Harris and Lewis (*6*), respectively.

The other measure of persistence, the number of eggs laid during August and September of the year following the date of hatch, was found to be a better measure of persistence than the number of eggs laid during the last 50 days of the laying year, as shown by the data in table 1.

There are evidently no highly significant correlations in either breed between the number of eggs laid during August and September and the date of hatch, date of first egg, and sexual maturity. This measure of persistence is highly and significantly correlated with percentage of production to March 1 in both breeds, the simple correlation coefficients being +0.252 and +0.149 and the partial correlations +0.078 and +0.164 for the White Leghorns and the Rhode Island Reds, respectively. This may be due to the linkage of some of the major genes for egg production, but it is more likely to be due to the fact that percentage of production is calculated from a part of the whole (egg production), and persistence is measured as a part of the same whole, and both, in a way, measure the same trait at different periods. The number of eggs laid during August and September may be considered as an expression of rate late in the laying year, as well as persistence. For instance, a bird that lays 30 eggs during this period would have a late rate of approximately 50 percent. It would be natural, therefore, to expect some correlation between number of eggs laid during August and September and percentage of production to March 1. As a matter of fact, it is surprising that the correlation coefficient between them is so low. Furthermore, the partial correlation coefficient between persistence and egg production, which includes percentage of production to March 1 as one of the

variables, is higher than the simple correlation coefficient, being +0.733 for the White Leghorns and +0.772 for the Rhode Island Reds. This would indicate that even when the percentage of production to March 1 is used as one of the variables there was no lowering of the correlation between the number of eggs laid during August and September and total egg production.

The data in table 3 show the correlation coefficients between persistence and various factors, obtained by Hays and Sanborn (*11*) and the present authors. Since the simple correlation coefficients between the number of eggs the last 50 days and Hays and Sanborn's measure of persistence are not significantly different, the two measures may be considered the same. These correlations were obtained even though the Rhode Island Red population (signifying both number of birds and various strains) was different in each case and different years were involved. The data in this table show also that the number of eggs laid during August and September is the only measure of persistence that lacks a significant correlation with sexual maturity, and it has the lowest correlation with the date of hatch and with sexual maturity. Hence, the number of eggs laid during August and September evidently is the best measure of persistence.

TABLE 3.—*Comparison of correlation coefficients between different measures of persistence and the factors indicated, for Rhode Island Reds*

Item of comparison	Investigators and measure of persistence used		
	Hays and Sanborn [1]	Knox, Jull, and Quinn	
		Last 50 days	August and September
Simple correlation coefficient between persistence and—			
Total egg production	+0.708	+0.667	+0.652
Percentage of production to Mar. 1	+.184	+.150	+.149
Sexual maturity	−.615	−.340	−.015
Date of hatch	−.221	−.212	+.078

[1] Their measure of persistence was a class interval of 15 days with a range of from 67 to 366 days.

The evidence presented does not necessarily show that linkage exists between sexual maturity and persistence, for the relation between the two might be due to an inadequate measure of persistence. This line of reasoning would invalidate the use of the low partial correlation coefficients reported by Hays and Sanborn (*11*) between sexual maturity and total production because of the high simple correlation between sexual maturity and the measure of persistence used.

MULTIPLE CORRELATIONS

In considering the problem from the standpoint of a multiple correlation study, the authors formulated table 4. The method of presentation is predicated, in part, on the deductions of Ezekiel (*4, p. 261*), who states:

* * * it is evident that the reliability of a regression coefficient varies directly with the multiple correlation of the dependent factor with the other factors, but inversely with the multiple correlation of the particular independent

factor with the other independents. The more closely a particular independent factor can be estimated from the other factors present, the less accurately can the net relation of the dependent factor to it be determined.

In calculating R and $\bar{R}$ in table 4, the authors used total egg production as the dependent variable with six independent variables. R represents the multiple correlation coefficient, and multiple $\bar{R}$ the most probable correlation for the fowls of the universe. Date of hatch, date of first egg, sexual maturity, and winter pause were used in each of the correlations. The other two independent variables consisted of some measure of rate and persistence, as shown in table 4. Various measures of rate and persistence were substituted for others to determine whether the different measures would improve the prediction value.

TABLE 4.—*Multiple correlations of six variables with total egg production*

Four variables [1] and—	White Leghorns		Rhode Island Reds	
	R	$\bar{R}$	R	$\bar{R}$
Number of eggs August and September; percentage of production to Mar. 1	0.874	0.873	0.887	0.886
Number of eggs August and September; number of eggs to Mar. 1	.876	.875	.894	.893
Number of eggs August and September; number of eggs first 50 days	.846	.844	.865	.864
Number of eggs last 50 days; percentage of production to Mar. 1	.841	.840	.855	.854

[1] Date of hatch, date of first egg, sexual maturity, and winter pause.

The table shows that there is little difference between the multiple R and $\bar{R}$ for the different measures substituted. In only one case was the difference significant. When the number of eggs laid in August and September was substituted for the number laid during the last 50 days, the $\bar{R}$ was increased from 0.840 to 0.873 in the case of the Leghorns and from 0.854 to 0.886 in the case of the Rhode Island Reds. As the value of p is between 0.01 and 0.02, the difference between the $\bar{R}$'s was significant in both breeds. The number of eggs laid in August and September, therefore, is the more valuable measure of persistence.

Since there is no material difference in the multiple correlation coefficients when different measures of rate are used, as shown in table 4, it is evident that they are measures of the same trait. Hence, from the discussion of the various simple, corrected partial, and multiple correlation coefficients, the following statements may be made: It is evident that when the date of hatch and the date of first egg occur at the proper time and within the limits of this experiment, they have no significant effect upon total egg production. Furthermore, the best measures of rate and persistence to use are the percentage of production before March 1 and the number of eggs laid during August and September of the year following the date of hatch. These two measures may be considered as hereditary characteristics, as is sexual maturity. From the foregoing evidence it would be expected that a multiple correlation coefficient between total egg production and the three hereditary characteristics just mentioned would be practically of the same magnitude as the highest of those shown in table 4, which include six independent variables.

Therefore, the multiple correlation coefficients between total egg production and the three major factors, sexual maturity, percentage of production to March 1, and eggs laid during August and September, were calculated. For the White Leghorns the R and $\overline{R}$ were each found to be 0.866; for the Rhode Island Reds, 0.887 and 0.886, respectively. For each breed the highest multiple correlation coefficient (R) is not materially different from the highest one given in table 4. The multiple correlation coefficient for the universe ($\overline{R}$) in the Rhode Island Reds, 0.886, is the same as for the same variables used in table 4, which included, in addition, the variables date of hatch, date of first egg, and winter pause. This correlation ($\overline{R}$) for the White Leghorn pullets is not significantly different. It is evident, therefore, that there is little to be gained by the addition of any more independent variables studied than the three major ones previously mentioned.

That these major variables add significant amounts to correlation is shown by the following: For the White Leghorns and Rhode Island Reds the simple correlation between number of eggs laid during August and September and total egg production is 0.692 and 0.652, respectively. When percentage of production to March 1 is added in a multiple correlation study, the multiple correlation coefficient is 0.839 and 0.850, respectively. The further addition of sexual maturity increases the coefficient to 0.866 and 0.886, respectively.

BROODINESS

Correlations were obtained between broodiness in Rhode Island Reds and various egg-production factors. The simple correlation with sexual maturity was +0.208; with percentage of production to March 1, −0.148; with number of eggs laid during August and September of the year following date of hatch, −0.156; and with total production, −0.342. Between broodiness and total production the corrected partial correlation coefficient was −0.260, R was 0.894, and $\overline{R}$ was 0.894, when sexual maturity, percentage of production to March 1, and the number of eggs laid during August and September were considered as additional variables. Because there was little broodiness in the White Leghorns, their records were not used in the correlation coefficient studies.

All the correlation coefficients are significant. They apparently indicate that later maturing birds are more likely to be broody than early maturing birds and that broodiness tends to reduce the rate and persistence of production. It may be that the correlation between broodiness and sexual maturity indicates some linkage between them. In any event, it would tend to complicate the interpretation of the inheritance of broodiness. Although the multiple correlation (0.894) is higher than the correlation (0.887) between total egg production and the three major factors previously mentioned, the difference is not significant. This may be due to selection over a period of years against broodiness, which would tend to lower the correlation.

The data of Hays and Sanborn (*12*) agree with the data of the authors with the exception of the low correlation which they obtained between broodiness and their particular measure of persistence. The differences between the findings of the authors and of Hays and Sanborn may be due to different populations and selection of nonbroody birds over a long period of time.

Hays and Sanborn (*12*) obtained a multiple correlation of 0.864 between egg production in Rhode Island Red pullets and five traits, but they used different measures of rate and persistence than were used in this study. The present authors obtained a multiple correlation of 0.894 for the Rhode Island Red data when four independent variables were used, and 0.887 when only three variables were used. The difference between the two coefficients 0.894 and 0.864 is highly significant, since the value of t is greater than 3. Even if the difference were insignificant, it would indicate that the addition of other measures, which are more or less duplicates, add practically nothing to the multiple correlation.

SUMMARY AND CONCLUSIONS

The records of 903 Rhode Island Red pullets and 884 Single-Comb White Leghorn pullets were used in 1928, 1929, and 1930 to ascertain the influence of the date of hatch and the date of first egg upon egg production during the first year, to find adequate criteria for predicting or estimating relative egg production, and to establish any linkages that might exist as indicated by the correlation coefficients.

It was found that the date of hatch and date of first egg have an insignificant effect upon egg production when the date of hatch is during the normal hatching season and of 7 or 8 weeks' duration, the period covered by this study. This fact was ascertained through the use of partial correlation coefficients. Between date of hatch and egg production these coefficients were +0.042 for the White Leghorns and −0.054 for the Rhode Island Reds, and between date of first egg and egg production they were −0.016 for the White Leghorns and −0.055 for the Rhode Island Reds.

The following known measures of hereditary traits that control egg production were used in this study: Sexual maturity (age at first egg), number of eggs laid the first 50 days, number of eggs laid to March 1, length of winter pause, percentage of production to March 1, number of eggs laid during August and September of the year following date of hatch, number of eggs laid the last 50 days of the first laying year, and broodiness.

Between sexual maturity and total egg production, the partial correlation coefficients, which were −0.398 for the White Leghorns and −0.479 for the Rhode Island Reds, were highly significant even though percentage of production to March 1, length of winter pause, and the number of eggs laid during August and September were included as the other variables.

It was found that the number of eggs laid the first 50 days, the number of eggs to March 1, length of winter pause, and percentage of production to March 1 were all measures of the same trait, rate of production. The best of these measures was found to be the percentage of production to March 1. This measure of rate had the smallest correlation coefficient with each of the environmental factors studied and, with the exception of the number of eggs the first 50 days, with sexual maturity also. In addition, the percentage of production to March 1 had a high partial correlation coefficient with egg production, +0.555 and +0.568 for the White Leghorns and Rhode Island Reds, respectively.

The number of eggs laid during August and September of the year following the date of hatch and the number of eggs laid the last 50

days of the first laying year were found to be similar measures of persistence of production. The better of these two measures was the number of eggs laid during August and September. The partial correlation coefficients between this measure of persistence and egg production were +0.733 for the White Leghorns and +0.772 for the Rhode Island Reds, these coefficients being higher than those between the other measure of persistence and egg production. The number of eggs laid during August and September had a much smaller correlation coefficient with each of the environmental and hereditary factors considered, except length of winter pause and percentage of production to March 1, than the number of eggs laid the last 50 days.

Broodiness was studied in the Rhode Island Red pullets only, as very little broodiness was encountered in the White Leghorns that were used. A partial correlation coefficient of −0.260 was obtained between broodiness and egg production. It was found also that the broody hens, on an average, matured later, laid at a slower rate, and were less persistent in production than the nonbroody hens.

The three major hereditary traits—sexual maturity, rate as measured by percentage of production to March 1, and persistence as measured by number of eggs laid during August and September—had a high multiple correlation with first-year egg production in both breeds. The multiple correlation coefficients, R, were 0.866 and 0.886 for the White Leghorns and Rhode Island Reds, respectively. When the fourth trait, broodiness, was added to these major traits in a multiple correlation study in the Rhode Island Reds, the coefficient, R, was found to be 0.894. Other factors that were studied, when added to the three major traits mentioned, produced no significant change in the size of the multiple correlation coefficient.

No evidence of linkage was found in either breed between the best measure of rate—percentage of production to March 1—and sexual maturity, as indicated by the coefficients. The simple correlation coefficients were very insignificant, being +0.022 and −0.015 for the White Leghorns and Rhode Island Reds, respectively. The simple correlation coefficients between the better measure of persistence—the number of eggs laid during August and September—and sexual maturity were −0.093 and −0.015 for the White Leghorns and Rhode Island Reds, respectively, indicating that there was comparatively little significance or linkage between these factors.

Partial correlation coefficients of +0.078 in the case of the White Leghorns and +0.164 in the Rhode Island Reds were found between percentage of production to March 1 and the number of eggs laid during August and September. These significant correlations are probably due to the fact that these two traits are measures of different parts of the whole (first-year egg production) and in a way are measures of the same thing at different periods, rather than being due to any linkage relationship. Even though they are calculated from different parts of the whole, the partial correlation coefficients are low enough to permit the use of both rate and persistence as criteria of egg production.

The simple correlation coefficients between the number of eggs laid during August and September and first-year egg production were +0.692 for the White Leghorns and +0.652 for the Rhode Islands Reds. When the multiple correlation coefficients were obtained between the number of eggs laid during August and September, the

percentage of production to March 1, and total egg production, the last-mentioned factor being used as the dependent variable, the coefficients obtained were 0.839 and 0.850 for the White Leghorns and Rhode Island Reds, respectively. The differences between the simple and multiple correlation coefficients were 0.147 and 0.198, and are highly significant. Therefore, as the percentage of production to March 1 adds a significant amount to the simple correlation coefficient between the number of eggs laid during August and September and egg production, it is desirable that both percentage of production to March 1 and the number of eggs laid during August and September be included in statistical and genetic investigations of egg production.

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CYTOGENETICS OF TETRAPLOID MAIZE[1]

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INTRODUCTION

Tetraploidy is unknown in normal stocks of maize, although large numbers of individuals have been examined cytologically and inspected under field conditions for the purpose of detecting tetraploids. In other plant genera, such as *Oenothera*, *Datura*, and *Primula*, which have been used extensively in breeding experiments, tetraploid individuals have been discovered at infrequent intervals. These tetraploid mutants with twice as many chromosomes as the normal diploids ordinarily exhibit gigas, or giant characteristics. Plant species with chromosome numbers in multiples of some base number such as 5, 7, 9, etc., are of wide-spread occurrence in nature. This fact suggests that chromosome doubling has played an important part in the evolution of new species.

The experimental production of tetraploid maize (*Zea mays* L.) was accomplished by subjecting plants in the early embryonic stage to high temperatures for short periods.[2] The treatments of the young ears were carefully timed to coincide with the first cell divisions in the development of the proembryo from the zygote. This was done in order to obtain individuals that would be entirely tetraploid.[3] Tetraploids were induced by the heat treatments with a frequency ranging from 2 to 5 percent. In addition, aborted, scarred, and defective grains and grains with mosaic endosperms were produced on the treated ears (fig. 1).

APPEARANCE OF TETRAPLOIDS

The giant characteristics typical of most tetraploids are only moderately developed in the vegetative organs of maize tetraploids. But the structures associated with the process of reproduction are definitely enlarged. In general appearance maize tetraploids do not differ markedly from the related diploid plants (fig. 2). They are of about the same height and have a similar habit of growth. However, their stalks are thicker and sturdier and the leaves are somewhat broader and thicker. The tassels also are larger and the individual parts of the staminate inflorescence are conspicuously enlarged. Abundant

[1] Received for publication Nov. 27, 1934; issued June 1935. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Department of Botany, New York State College of Agriculture, Cornell University. This investigation was aided by a grant from the committee on effects of radiation upon living organisms, of the Division of Biology and Agriculture, National Research Council.

[2] RANDOLPH, L. F. SOME EFFECTS OF HIGH TEMPERATURE ON POLYPLOIDY AND OTHER VARIATIONS IN MAIZE. Natl. Acad. Sci. Proc. 18: 222-229. 1932.

[3] RHOADES, V. H. A STUDY OF FERTILIZATION IN ZEA MAYS. (Master's thesis, Cornell Univ.) 1934. [In manuscript.] Rhoades determined the time interval from pollination to fertilization in maize at a constant temperature of 25° C. With silk lengths of 7 to 11 cm the interval was 15 hours; with silks 11 to 14 cm long the interval was 17 hours. The primary endosperm nucleus was found to divide almost immediately after fertilization, and 4 to 8 endosperm nuclei were present at the time of the division of the zygote 10 to 12 hours after fertilization.

pollen of uniform size but much larger than that of diploids is produced by the $4n$ plants (fig. 3, *A*, *B*). From the seedling stage to maturity the $4n$, as compared with the $2n$ individuals of the same stage in development, have larger and more widely spaced stomata and correspondingly larger epidermal cells (fig. 3, *C*, *D*). These differences serve as useful criteria for identifying the tetraploids.

The ears and kernels of tetraploid maize are distinctly larger than those of comparable diploid stocks. This fact was clearly demonstrated by an experiment in which tetraploidy was induced in the F_1 hybrid

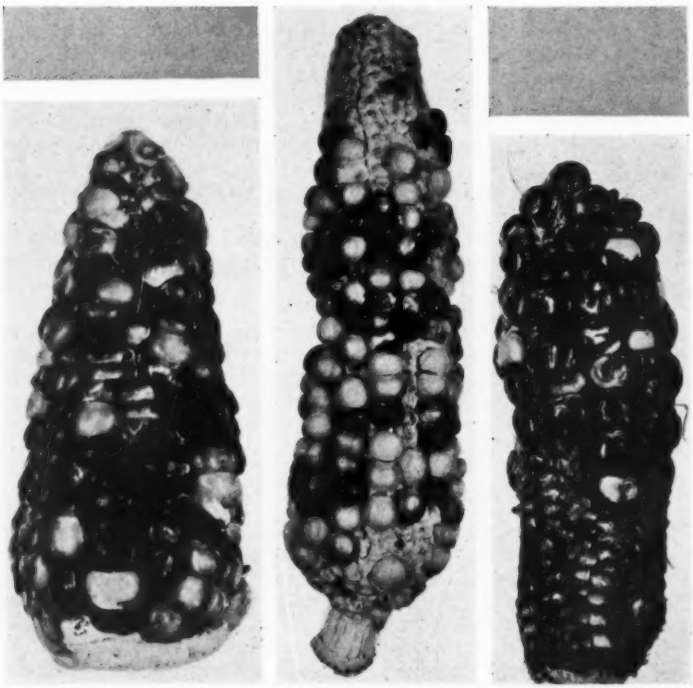

FIGURE 1.—Mature ears of maize treated with heat shortly after fertilization to induce tetraploidy. In addition to the production of tetraploids, the normal frequency of defective, scarred, and mosaic grains also was markedly increased by the heat treatments.

between two similar inbred lines and in which a comparison was made between self-pollinated tetraploid and diploid lines derived from the induced tetraploids and their diploid sibs. In figure 4 are illustrated ears that are representative of the parental diploid lines, the F_1 diploid hybrid, and a derived tetraploid line.

To obtain an indication of the relative weights of the $2n$ and $4n$ kernels in these lines, several samples of shelled grain, each containing 1,000 kernels, were weighed. The average weight of three such diploid samples was 221.6 g; the average for the same number of tetraploid samples was 330.2 g, or approximately 50 percent more. However, the $4n$ ears were not so well filled as those of the diploid,

and consequently the kernels were less crowded on the ear, a condition which probably accounted for part of their increased weight. In figure 5 are shown samples of the shelled grain from the lines used in making the weight comparisons.

FIGURE 2.—Normal diploid (*A*) and induced tetraploid (*B*) sister maize plants from the progeny of heat-treated ears.

BREEDING BEHAVIOR

When selfed or intercrossed, maize tetraploids produce large ears with large, well-filled kernels. Partially developed or defective kernels are rarely formed. Pollen is shed freely, and ordinarily $4n$ plants produce no more shriveled or aborted pollen than the 3 to 5 percent normally produced by diploid stocks. The fertility of the

tetraploids is somewhat reduced, as indicated by the presence of aborted ovules and an irregular spacing of the kernels on the mature ears. In comparison with diploid stocks the amount of reduced fertility varied from 5 to 20 percent in the $4n$ stocks thus far produced.

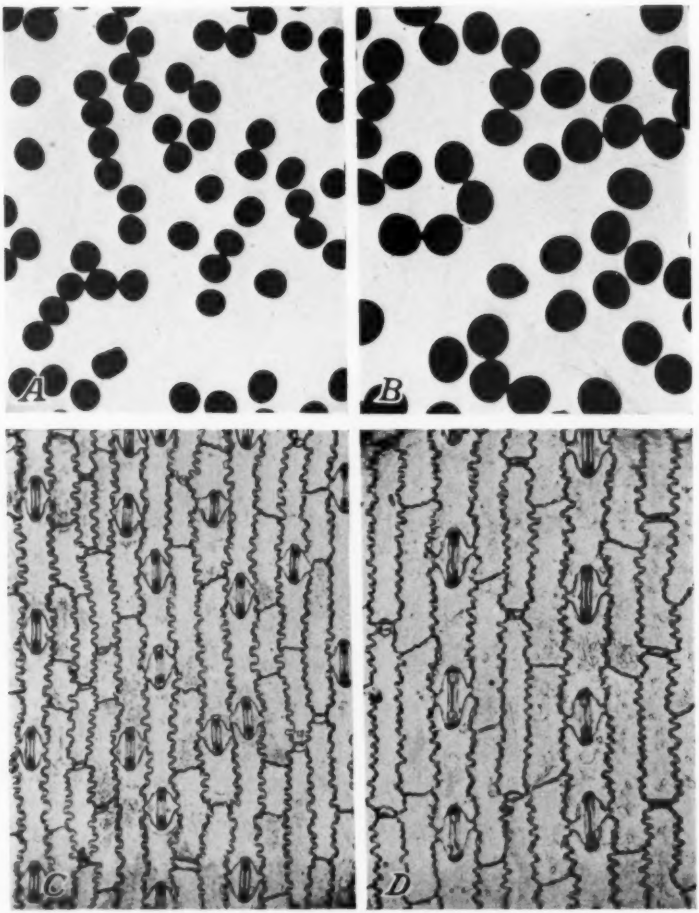

FIGURE 3.—Photomicrographs of the pollen of diploid (*A*) and tetraploid (*B*) maize, and of the lower epidermis of the leaves of mature diploid (*C*) and tetraploid (*D*) maize. × 55.

In exceptional cases a higher percentage was found in individuals which also had some aborted pollen. This type of infertility apparently is not due to the doubling of the chromosomes, since it occurs in both the diploid and tetraploid progenies of heat-treated plants.

Tetraploid stocks breed true for tetraploidy with only slight deviations in chromosome number in subsequent generations. Mater-

nal diploids, which are to be expected at infrequent intervals in the progeny of tetraploids, provided parthenogenesis occurs in tetraploids as in diploids, thus far have not been observed.

Since tetraploids have 4 sets instead of 2 sets of homologous chromosomes, the Mendelian ratios obtained from them are very different from those obtained from diploids.[4] The selfed ears from

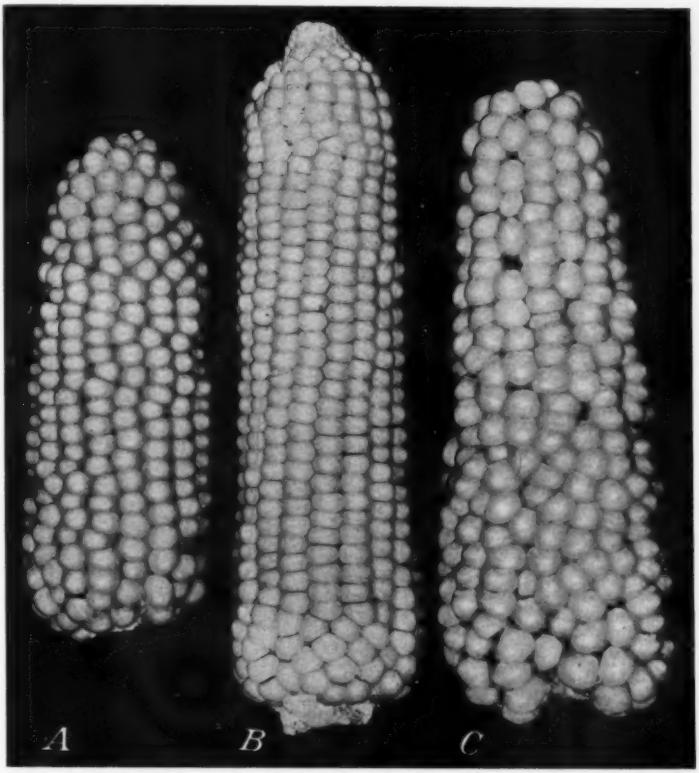

FIGURE 4.—Representative ears of an inbred line (*A*), the F_1 hybrid (*B*) between line *A* and a similar line, and a tetraploid line (*C*) derived from the F_1 hybrid and inbred for two generations.

$2n$ and $4n$ plants shown in figure 6 illustrate typical diploid and tetraploid F_2 segregations for the aleurone factor pair *Rr*. They approximate the expected 3:1 and 35:1 ratios. Similar segregations for other seed, seedling, and mature plant characters also have been noted in tetraploid maize stocks, but the data are inadequate to determine whether or not there are significant deviations from the expected ratios.

[4] BLAKESLEE, A. F., BELLING, J., and FARNHAM, M. E. INHERITANCE IN TETRAPLOID DATURAS. Bot. Gaz. 76: 329–373. 1923.
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TETRAPLOID AND DIPLOID INTERCROSSES

The intercrosses between diploid and tetraploid maize exhibited a high degree of sterility in both direct and reciprocal crosses. The resulting progeny with rare exceptions were triploid hybrids. When the diploid was the seed parent the fertility, as measured by the proportion of the viable to the aborted and defective seed, was less than 0.5 percent. When the tetraploid was the seed parent there was approximately 5 percent fertility. Mixtures of approximately equal amounts of pollen from $4n$ and $2n$ stocks applied to the silks of the $4n$ and $2n$ parent plants resulted in a set of seed by the diploid almost exclusively from its own pollen; the tetraploid, however, set very little seed from its own pollen, the majority being defective kernels from the pollen of the diploid. The pollen of the tetraploid apparently

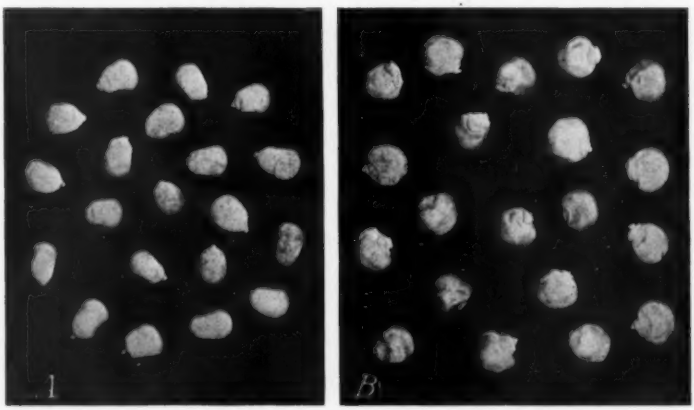

FIGURE 5.—Diploid (*A*) and tetraploid (*B*) kernels of maize from 2-generation inbred $2n$ and $4n$ lines derived from an F_1 hybrid between inbred strains with kernels of a similar type.

was unable to compete favorably with that of the diploid either on its own silks or on those of the diploid.

The crossing behavior of tetraploid and diploid strains was very carefully studied under field conditions in an isolated plot, in order to exclude the possibility of contamination from plants other than those used in the experiment. In this crossing experiment special attention also was given to the selection of strains with combinations of genetic characters that would facilitate not only the determination of the amount of natural crossing but also the positive identification of the various possible types of progeny. Within the plot $4n$ and $2n$ plants were grown in alternate rows to insure abundant opportunity for natural cross-pollination. To verify the results obtained from the open-pollinated ears, controlled pollinations were made with separate and mixed samples of pollen from the two stocks.

The $4n$ stock selected for the crossing studies was a green plant type, lacking anthocyanin color (*A b pl* r^g) and having nonliguleless leaves (*Lg*), nonyellow endosperm (*y*), and colorless aleurone (*A C r*)[5].

[5] EMERSON, R. A. A FIFTH PAIR OF FACTORS, Aa, FOR ALEURONE COLOR IN MAIZE, AND ITS RELATION TO THE Cc AND Rr PAIRS. N. Y. (Cornell) Agr. Expt. Sta. Mem. 16, pp. 231–289, illus. 1918.
——— THE GENETIC RELATIONS OF PLANT COLORS IN MAIZE. N. Y. (Cornell) Agr. Expt. Sta. Mem. 39 156 pp., illus. 1921.

The $2n$ stock was also a green plant type, but it had a genetic constitution (*a B pl* R^r) differing from that of the $4n$ stock, so that the F_1 hybrid would have sun-red plant color and colored anthers, phenotypically *A B pl* R^r and readily distinguishable from both parent types. The

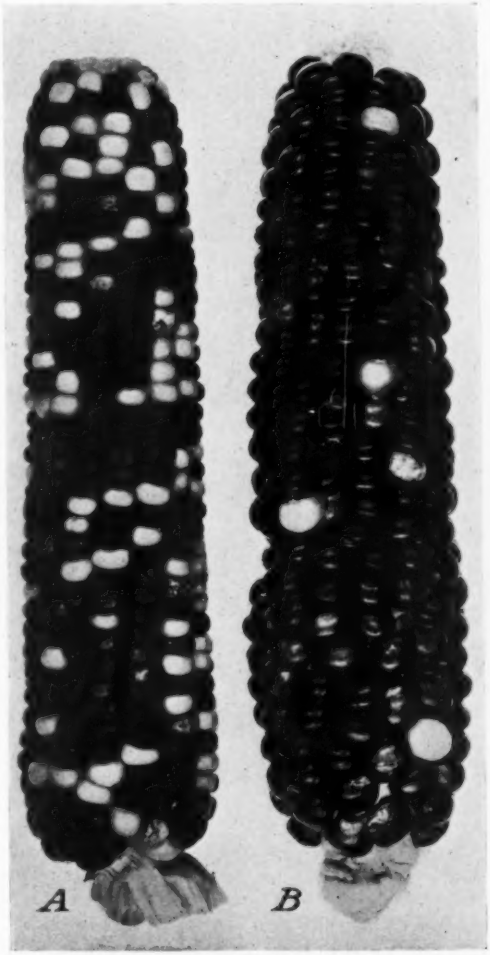

FIGURE 6.—Selfed ears of diploid (*A*) and tetraploid (*B*) maize plants heterozygous for the factor pair *Rr* illustrating segregations that approximate the expected 3 : 1 and 35 : 1 ratios.

$2n$ stock, moreover, was liguleless (*lg*), with yellow endosperm (*Y*) and colorless aleurone of a constitution (*a C R*) that would give colored aleurone and yellow endosperm in hybrid combinations with the $4n$ stock.

With these combinations of genes the selfed seed on both the $2n$ and $4n$ plants would be colorless and the hybrid seed colored. Assuming normal fertilization, the colorless seed would produce only green plants. Irregularities in the fertilization process, resulting in maternal embryos developed parthenogenetically or from heterofertilization,[6] would be detectable as green plants of the parental types appearing among the hybrid sun-red plants from the colored seeds.

Representative ears of the parent stocks and their hybrids are illustrated in figure 7. Few fully developed kernels were produced on the cross-pollinated ears, the majority of the grains being only partially developed. This partial development, however, involved the endosperm as well as the pericarp, so that the defective hybrid grains could be distinguished from the nonhybrid grains on the basis of aleurone color. The results obtained from intercrossing the $2n$ and $4n$ stocks are summarized in table 1. The hybrid seeds both from the direct and the reciprocal cross were roughly classified as well filled and aborted. Many of the well-filled grains, i. e., grains with fully developed endosperms, were germless or had defective germs and failed to produce viable seedlings. In the cross $2n \times 4n$ only about 10 percent of the well-filled grains and very few of the aborted grains germinated. In the reciprocal $4n \times 2n$ cross nearly all of the well-filled grains and a large number of the aborted grains produced viable seedlings. All of the seedling progeny of these crosses were triploid with the exception of four tetraploid seedlings from one $2n$ ear. Presumably this ear had a $4n$ sector which produced $2n$ eggs, these in combination with normal $2n$ male gametes from the $4n$ pollen parent giving the exceptional tetraploids.

TABLE 1.—*Intercrosses between diploid (a C R^r B–lg) and tetraploid (A C r^g b–Lg) strains of maize grown in an isolated plot and hand-pollinated*

Type of cross	Ears pollinated	Hybrid (colored) seeds		Seedling progeny (sun-red, nonliguleless)	
		Well filled	Aborted	3*n*	4*n*
	Number	*Number*	*Number*	*Number*	*Number*
$2n \times 4n$	253	427	20,180	58	4
$4n \times 2n$	96	90	10,170	[1] 438	

[1] Included in this number are 2 exceptional plants which were nonliguleless but lacked plant color.

All but 2 of the 500 F_1 seedlings from the $2n$ and $4n$ intercrosses were phenotypically sun-red and nonliguleless, indicating that they were true hybrids of constitution *Aa Bb–Lg!g* R^r r^g. Chromosome counts made from 150 of these sun-red nonliguleless seedlings, selected at random from the entire group of 498, showed that all were triploid. The 2 exceptional seedlings were nonliguleless and lacked plant color in the seedling stage. At maturity the glumes and anthers also were green, indicating the phenotypic constitution *A b Lg* r^g. Both of these plants were 30-chromosome triploids, as determined by root-tip counts, and their triploid nature was verified by pollen and stomata examinations at maturity. The absence of

[6] SPRAGUE, G. F. THE NATURE AND EXTENT OF HETERO-FERTILIZATION IN MAIZE. Genetics 17: 358-368, illus. 1932.

the characters normally associated with the nonlinked dominant genes *B* and *R*^{r} in these two plants is not understood. However, cogent cytogenetic data may be obtained from their progeny. No

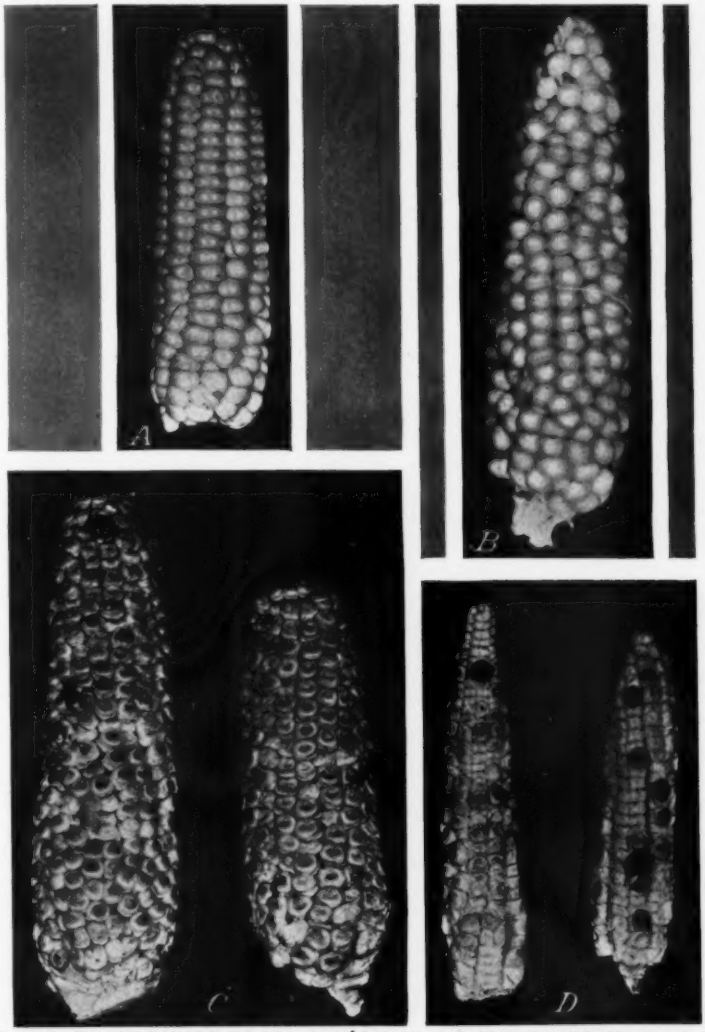

FIGURE 7.—Ears of diploid *a C R*^{r} *B-lg* (*A*) and tetraploid *A C r*^{g} *b-Lg* (*B*) stocks and the direct 2*n*×4*n* (*D*) and reciprocal 4*n*×2*n* (*C*) crosses between them.

other cytogenetically detectable exceptional types, such as maternal monoploids or diploids, appeared in the 2*n* and 4*n* crosses.

The crossing behavior of the 2*n* and 4*n* stocks grown together in a mixed isolated planting and open-pollinated was compared with con-

trolled pollination. Approximately equal amounts of pollen from the $2n$ and $4n$ stocks were thoroughly mixed and applied to both the $2n$ and $4n$ parent plants in the isolated plot. The results are summarized in table 2. Representative ears showing the set of seed obtained from these mixed pollinations are illustrated in figure 8. In this, as in the former experiment, the selfed seeds were colorless and the crossed seeds were colored.

TABLE 2.—*Intercrosses between diploid (a C R^r B–lg) and tetraploid (A C r^o b–Lg) strains of maize grown together in an isolated plot, comparing open pollinations and hand pollinations with mixtures of 2n and 4n pollen*

Type of cross	Ears pollinated	Crossed seed (colored)		Selfed seed (non-colored)
		Well filled	Aborted	
	Number	*Number*	*Number*	*Number*
$2n \times (2n+4n)$	77	5	103	9,938
$4n \times (2n+4n)$	26	60	3,324	467
$2n$ (open pollinated)	105	7	23	18,200
$4n$ (open pollinated)	81	79	8,311	8,148

In the cross $2n \times (2n+4n)$ the pollen of the diploid functioned almost to the exclusion of the $4n$ and produced uniformly well-filled ears of colorless selfed seed with only an occasional colored hybrid grain (fig. 8, *A*). The cross $4n \times (2n+4n)$ produced chiefly hybrid grains most of which were aborted; relatively few (5 to 10 percent) selfed seed were formed when the cross was made with the tetraploid as pistillate parent (fig. 8, *B*). Similar results were obtained with the open-pollinated ears. Most of the $4n$ ears showed considerable evidence of hybridization, the ratio of selfed to hybrid seed probably being dependent in large measure upon the relative amounts of $2n$ and $4n$ pollen available when the silks were exposed (fig. 8, *C*). However, the diploid ears under similar conditions showed little or no evidence of hybridization (fig. 8, *D*). The mixed open and closed pollinations recorded in table 2 involved 289 ears, which produced 48,665 kernels. From the hybrid colored seed there were obtained 214 seedlings, of which 16 were from diploid ears and the remainder from the tetraploid ears. All of these seedlings were triploid hybrids. The frequency with which hybrid seedling progeny resulted from the mixed pollinations was less than 1 percent.

These results indicate that $2n$ and $4n$ strains of maize are highly cross-sterile. Furthermore the triploid progeny of these crosses are themselves highly sterile (fig. 9), and yield mostly defective plants with unbalanced chromosome numbers. Thus tetraploid maize can be maintained under natural field conditions without hybridizing effectively with diploid stocks, a fact of major practical importance if it is found that tetraploid lines can be utilized advantageously in the production of hybrid seed corn.

The high degree of incompatibility exhibited by the maize tetraploids in crosses with normal diploid stocks obviously is to be attributed to quantitative rather than qualitative differences—i. e., to chromosome number differences—since the derived $4n$ stocks obtained in the experiments had a genetic constitution either very similar to or identical with that of the parental diploid stocks. Triploid

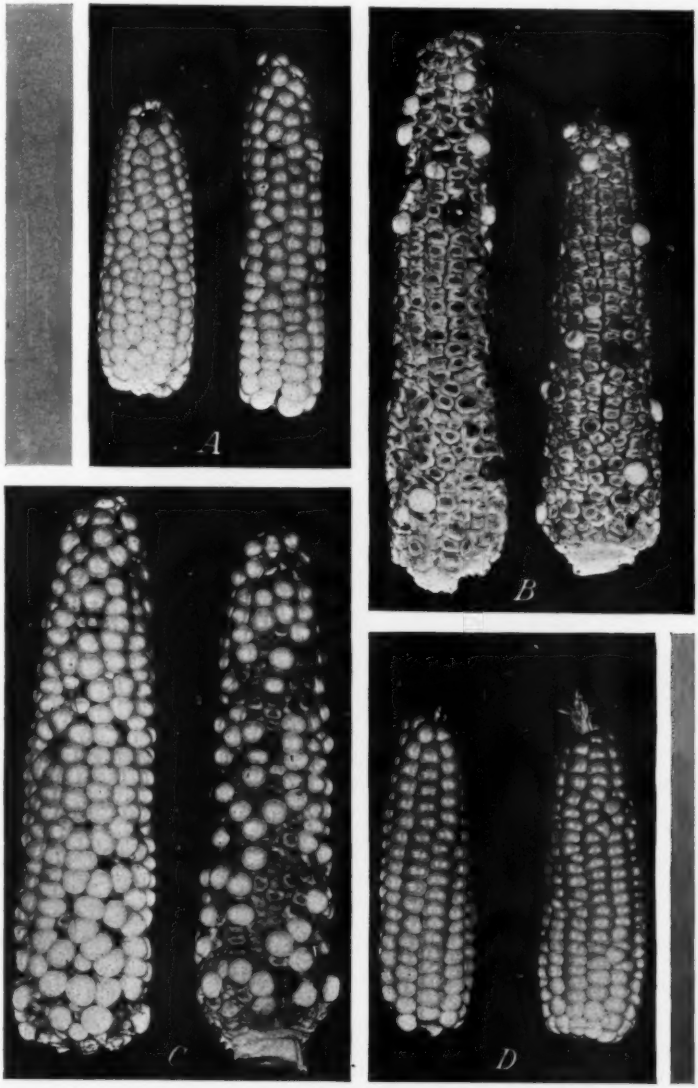

FIGURE 8.—Ears illustrating the set of seed obtained from intercrossing diploid *a C R* (*A*) and tetraploid *A C r* (*B*) stocks with mixtures of pollen from the two parents; also open-pollinated tetraploid (*C*) and diploid (*D*) ears of these same stocks grown together in an isolated plot.

hybrids were obtained with a low frequency in both direct $2n \times 4n$ and reciprocal $4n \times 2n$ crosses, the latter yielding a significantly higher frequency. Ineffectual pollen-tube growth is excluded as a cause of this incompatibility, since fertilization took place and kernel development uniformly was initiated, as evidenced by the presence of aborted

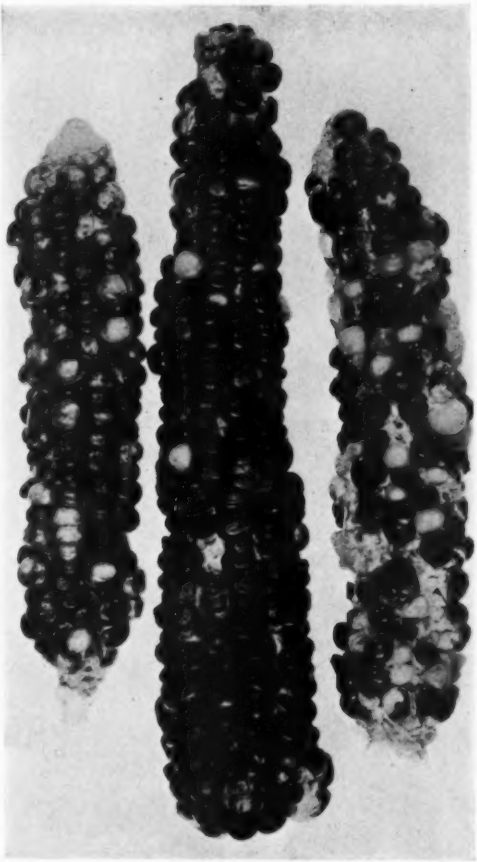

FIGURE 9.—Typical ears of self-pollinated triploid maize produced by intercrossing diploids and tetraploids.

hybrid grains. In fact, the results of the mixed and open $4n \times (2n+4n)$ pollinations demonstrated that the pollen of the diploid actually functioned on the $4n$ plants almost to the complete exclusion of the pollen of the tetraploid.

None of the current hypotheses advanced to account for the arrested seed development, or so-called "hybrid incompatibility" between autotetraploids and the parental diploids, when considered individually, offers a wholly satisfactory explanation of the situation in maize. It is possible that changed chromosome relationships between the embryo and endosperm, or between the embryo, endosperm, and

seed-parent tissue; or different strengths of activating stimuli in the male gametes, correlated with chromosome number and influencing the rate of growth of the embryo and endosperm, may account for part but certainly not for all of the observed facts pertaining to the direct and reciprocal crosses and to the occurrence of spontaneous triploids.

Noteworthy is the fact that the nonoccurrence of spontaneous tetraploids may be better understood in view of the observed inability of $2n$ pollen grains produced by tetraploids to compete successfully with the n pollen normally produced by diploids.

CHROMOSOME BEHAVIOR

The 40 chromosomes of tetraploid maize were arranged at the metaphase of the first meiotic mitosis usually in quadrivalent and bivalent groups exclusively (fig. 10, *A*). Trivalents and univalents

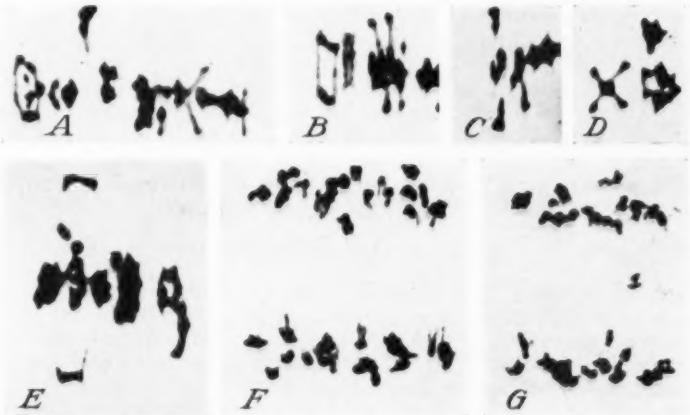

FIGURE 10.—Photomicrographs of the chromosomes of tetraploid maize illustrating certain phases of the first meiotic or reduction division: *A*, Metaphase I with quadrivalent and bivalent associations; *B–D*, early anaphases with adjacent (*B* and *D*) and alternate (*C*) members of certain quadrivalents passing to the same pole; *E*, precocious disjunction of one quadrivalent with adjacent members still attached; *F*, typical anaphase I with regular assortment of daughter chromosomes; *G*, anaphase I with a single lagging univalent chromosome.

were noted very rarely, the frequency being 11 out of a total of 402 metaphases observed. Most frequently the number of quadrivalents varied from 7 to 9, and the number of bivalents varied correspondingly from 3 to 1. Characteristic types of quadrivalent associations are illustrated in figure 10, *A*, *D*, the arrangements being of such a nature that either adjacent (fig. 10, *B*) or alternate (fig. 10, *C*) chromosomes pass to the same pole in the anaphase. In the former case the adjacent chromosomes passing to the same pole frequently were not separated from one another until they were some distance from the equatorial plate region. The anaphase figures usually were regular, with 20 chromosomes passing to one pole and 20 to the other (fig. 10, *B*). In 14 of 21 anaphases counted, each group contained 20 chromosomes; in 6 cases the disjunction was 19 to 21 and in one case it was 18 to 22. A search was made for lagging univalent chromosomes in the mid and late anaphase and early telophase stages;

13 cases of a single lagging univalent (fig. 10, *G*) and 2 cases of 2 lagging univalents were noted among 348 such figures. In a survey of the first divisions in the microspore, 16 spores were found with 20 chromosomes, 5 with 19, 2 with 18, 4 with 21, and 1 with 22 chromosomes.

The observations on chromosome distribution at meiosis and the microspore counts suggested that the tetraploids probably would not breed true for chromosome number. The following counts were made from seedling root tips of plants selected at random from among the selfed progeny of individuals known to have 40 chromosomes:

Chromosome number:	*Number of individuals*
37	1
38	3
39	6
40	27
41	12
42	5

Half of the 54 plants counted had 40 chromosomes like the parent; the others had numbers other than 40, ranging from 37 to 42. From an examination of these plants at maturity there appeared to be no definite positive correlation between off-type chromosome number and deficient vigor. These data were obtained from first- and second-generation tetraploids. Whether the tendency to breed true for chromosome number tends to become more or less variable in subsequent generations is not known.

SUMMARY AND CONCLUSIONS

Tetraploid maize (*Zea mays* L.) closely resembles normal diploid maize in height and habit of growth. But the individual organs of tetraploid plants are larger than those of the parental diploids; broader leaves, sturdier stalks, larger tassels, and ears and kernels of increased size characterize the tetraploids.

An increase in cell size with no apparent diminution in chromosome size accompanies chromosome doubling in maize, with the result that the pollen, epidermal cells, and stomata, as well as the cells in other parts of $4n$ plants, are proportionately larger than those of the diploid plants from which they were derived.

The tetraploid strains of maize thus far produced are not as fertile as the original diploids, the ears ordinarily being not entirely well filled. Taking the set of seed as a measure of fertility, the amount of reduced fertility varies from 5 to 20 percent in different $4n$ stocks.

Diploid maize and tetraploid maize exhibit marked incompatibility and are highly cross-sterile in both direct and reciprocal crosses. When the diploid was used as the seed parent the amount of fertility as measured by the proportion of viable to nonviable seeds was less than 0.5 percent. In the reciprocal cross the amount was somewhat higher, ranging from 3 to 5 percent. The resulting progeny with rare exceptions were triploid hybrids.

The pollen of tetraploid plants is unable to compete favorably with that of $2n$ plants, either on its own silks or on the silks of diploids, as indicated by the results of open and controlled pollinations in an isolated mixed planting of $2n$ and $4n$ stocks.

Tetraploid maize can be maintained under natural field conditions together with ordinary diploid maize without an appreciable amount of hybridization.

The high degree of incompatibility exhibited by tetraploid maize in crosses with diploid maize is to be attributed to quantitative rather than to qualitative chromosomal differences, since the induced $4n$ stocks had the same or very similar kinds of genes as the parental diploids, with which they subsequently proved to be highly cross-sterile.

Tetraploid stocks breed true for tetraploidy, no reversions to diploidy having been observed during several generations of breeding.

Variations in chromosome number involving one or a few chromosomes more or less than the typical number 40 occur in the progeny of tetraploids. This variation in number apparently has little or no effect on the appearance of the plants. There was no definite positive correlation between atypical chromosome number and deficient vigor or unusual habit of growth.

In meiosis the association of the chromosomes was almost exclusively in quadrivalents and bivalents, with usually 7 to 9 quadrivalents and 3 to 1 bivalents; trivalents were rarely seen. Irregularities in meiotic chromosome distribution were noted which would account for the observed inconstancy of number in the progeny of tetraploids.

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COMPARATIVE RESISTANCE OF FIVE BREEDS OF CHICKENS TO THE NEMATODE ASCARIDIA LINEATA (SCHNEIDER)[1]

By J. E. ACKERT, *Kansas Agricultural Experiment Station parasitologist*, and L. L. EISENBRANDT, J. H. WILMOTH, B. GLADING, and I. PRATT, *graduate research assistants in parasitology, Kansas Agricultural Experiment Station*

INTRODUCTION

Experimental evidence of the resistance of chickens to the nematode *Ascaridia lineata* (Schneider) was obtained in 1921 by Ackert and Herrick (*9*) [2] while studying the effects of this intestinal parasite on chickens. This finding led to studies of the factors influencing this resistance. Among the significant factors were found to be the presence of vitamins A and B, certain dietary supplements, loss of blood, previous infestations, and age. The significance of vitamin A was reported by Ackert, McIlvaine, and Crawford (*10*); that of vitamin B, by Ackert and Nolf (*12*); that of certain dietary supplements, by Ackert and Beach (*4*); and that of loss of blood, by Ackert (*1*) and by Porter and Ackert (*25*). Graham, Ackert, and Jones (*18*) found that resistance was influenced by previous infestations of *A. lineata*, and Herrick (*20*) and Ackert, Porter, and Beach (*13*) that age was a factor. All these studies were made on purebred Single-Comb White Leghorns. As 2-month-old chicks of this breed were found by Ackert (*2*) to be very susceptible to *A. lineata*, it seemed desirable to ascertain whether other breeds of chickens were more resistant to this nematode than White Leghorns, or less.

Accordingly, experiments were begun in 1932 to compare the resistance of Single-Comb White Leghorns to *Ascaridia lineata* with that of Single-Comb Rhode Island Reds, Barred Plymouth Rocks, White Plymouth Rocks, Buff Orpingtons, and White Minorcas.

MATERIALS AND METHODS

The chickens used in these experiments were secured as day-old chicks from the same commercial hatchery and raised in confinement under conditions found by Herrick, Ackert, and Danheim (*21*) to be suitable for normal growth and development. In these experiments the following ration was used: Yellow corn meal, 40 g; ground oats, 17 g; alfalfa leaf meal, 4 g; meat meal, 10.4 g; powdered milk, 6.4 g; cracked wheat, 15 g; and cod-liver oil, 1.69 g. In order that the vitamin A potency of the cod-liver oil might be retained, a barrel of feed sufficient for 300 chickens was mixed each week.

The nematode eggs for parasitizing were secured from adult live worms of approximately the same length. The egg cultures were prepared in the following manner: The end of a worm was excised, and the internal organs were pressed into a sterile Petri dish; the uteri were isolated and transferred to another sterile dish. At various

[1] Received for publication Nov. 27, 1934; issued June 1935. Contribution No. 165 from the Department of Zoology, Agricultural Experiment Station, Kansas State College of Agriculture and AppliedScience.

[2] Reference is made by number (italic) to Literature Cited, p. —.

points the uteri were punctured for the liberation of eggs with characteristic light centers, shown by Ackert (*3*) to be fertile. The portions of uteri containing fertile eggs were transferred to a final sterile Petri dish, and the eggs pressed out and covered with sterile distilled water. Four or five drops of 2-percent formalin were added to prevent bacterial or fungous growth. The cultures were incubated approximately 3 weeks at 27° to 30° C.

When the chicks were 9 days old, they were banded, divided into groups, and each chick weighed. Weekly weights were taken thereafter until the termination of the experiment. Group A was parasitized at 12 days of age, group B at 19 days, group C at 30 days, group D at 33 days, and group E at 44 days. In parasitizing, each chicken of a group was given 50 ± 5 embryonated eggs of *Ascaridia lineata* as Ackert, Graham, Nolf, and Porter (*7*) found this number suitable for comparative infestations of the worms. The eggs were counted with the aid of a compound microscope and a mechanical stage. The drop of water containing the correct number of eggs was wiped off the slide with a small piece of filter paper, which was placed in the chick's esophagus with forceps.

After 3 weeks of parasitism the chickens were killed, the intestines removed, and the contents flushed out quickly by the hydraulic method of Ackert and Nolf (*11*). The nematodes were preserved in 10-percent formalin. In measuring, the shadow of each worm magnified six times, was thrown on the ground glass of a photographic bellows and traced on onionskin paper; from these tracings direct measurements were made with a calibrated, milled wheel.

The criteria adopted in making statistical comparisons of resistance among the different breeds of chickens to *Ascaridia lineata* were the number of worms and their length from each group of chickens under comparison.

EXPERIMENTAL DATA

GROWTH OF THE CHICKENS

In the various experiments 1,351 chickens were used. The average weights taken at 9 days of age showed the White Leghorns and White Minorcas, both Mediterranean breeds, to be the heaviest, slightly over 62 g each. The heavier breeds and varieties averaged distinctly less: The Rhode Island Reds, 53.6 g; the Barred Plymouth Rocks, 54.6 g; the White Plymouth Rocks, 57.1 g; and the Buff Orpingtons, 57.7 g (fig. 1). All the chickens were kept under the same conditions and were given the same ration, which was kept constantly before them. At the close of the first week of the experiment, the White Plymouth Rocks averaged the heaviest and the Barred Plymouth Rocks the lightest, the White Minorcas taking a lower rank and the Rhode Island Reds and Buff Orpingtons making more rapid gains. In 2 weeks, when the chicks were 30 days old, the White Plymouth Rocks were the heaviest, the White Leghorns next, the Buff Orpingtons and Rhode Island Reds following them, with the White Minorcas and Barred Plymouth Rocks making the least rapid gains. During the next 2 weeks the White Leghorns' rate of gain decreased and that of each of the heavier breeds or varieties, the White Plymouth Rocks, Buff Orpingtons, Rhode Island Reds, and Barred Plymouth Rocks, made better gains. The White Minorcas continued to make the slowest increase in weight (fig. 1).

At 51 days of age a slight break may be noticed in the growth rates of some of the breeds or varieties of chickens.

This result is in accord with that of Brody (*14*), who found two characteristic growth cycles in the post-embryonic life of domestic chickens, one of which climaxed at about 8 weeks of age.

After about a week, when the chickens were 58 days of age, an increased growth rate again appeared in all 5 breeds or varieties (fig. 1). At that time the White Leghorns and White Minorcas were definitely separated in their growth rates from the 3 other breeds or varieties, and 1 week later the White Leghorns had the slowest growth and the White Minorcas next. Of the 4 heavier breeds or varieties, the White Plymouth Rocks continued to lead and the Buff Orpingtons to trail in growth.

At the close of the experiment (end of the seventh week), when the chickens were 63 days of age, the White Plymouth Rocks were slightly the heaviest and the Rhode Island Reds next. Third and fourth in weight, respectively, were the Barred Plymouth Rocks and Buff Orpingtons. The White Minorcas, a breed shown by Jull (*22*) to be somewhat heavier than the White Leghorns, were making rapid growth (fig. 1).

The weights of these chickens grown in confinement but with ample light and air are very similar to those of corresponding breeds raised by Warren (*33*) at the Kansas State College Poultry Farm. Warren made weekly records of the weights of White Leghorns, Rhode Island Reds, and Barred Plymouth Rocks for 10 weeks, beginning with day-old chicks. At 6 weeks of age the weights of the White Leghorns and Rhode Island Reds in his and in the present experiments were almost identical. His Barred Plymouth Rocks, however, were somewhat smaller, averaging 274.2 g as compared with 334.6 g in the present experiments. Similar weights were recorded by Kempster (*23*) and by Card and Kirkpatrick (*15*) for corresponding breeds and varieties of chickens at 6 weeks of age.

THE RESISTANCE OF WHITE LEGHORNS COMPARED WITH THAT OF THE OTHER BREEDS AND VARIETIES

Group A, 33 Days of Age

The experiments on resistance were conducted in five groups, A to E. In group A, the chickens, when 12 days of age, were parasitized with 50 ± 5 eggs of the nematode *Ascaridia lineata*. After the 3-week period of parasitism all chickens of the group were killed, the worms from each bird counted and measured, and the data for each breed or variety assembled.

In table 1 it is seen that from the White Leghorns there was an average of 8.28 worms per bird and from the Rhode Island Reds an average of 4.89 worms, a difference of 3.39 worms which was 3.03 times the probable error and was therefore considered to be significant. In comparing the length of the worms from these two groups it was found that those from the Leghorns averaged 22.08 mm and the ones from the Reds 17.66 mm, a significant difference of 4.42 mm. The results of this test indicate that the Rhode Island Reds were significantly more resistant to *Ascaridia lineata* than were the White Leghorns.

The data in table 1 for the 36 White Plymouth Rocks indicate that they were more resistant than the Leghorns to the growth of the nematodes.

The Buff Orpingtons in this group proved to be just as susceptible as were the White Leghorns to *Ascaridia lineata*, the differences in number and in length having been negligible.

No constant difference was found between the number of nematodes from the Barred Plymouth Rocks and that from the Leghorns, but on comparing the length of the worms it was found that the worms

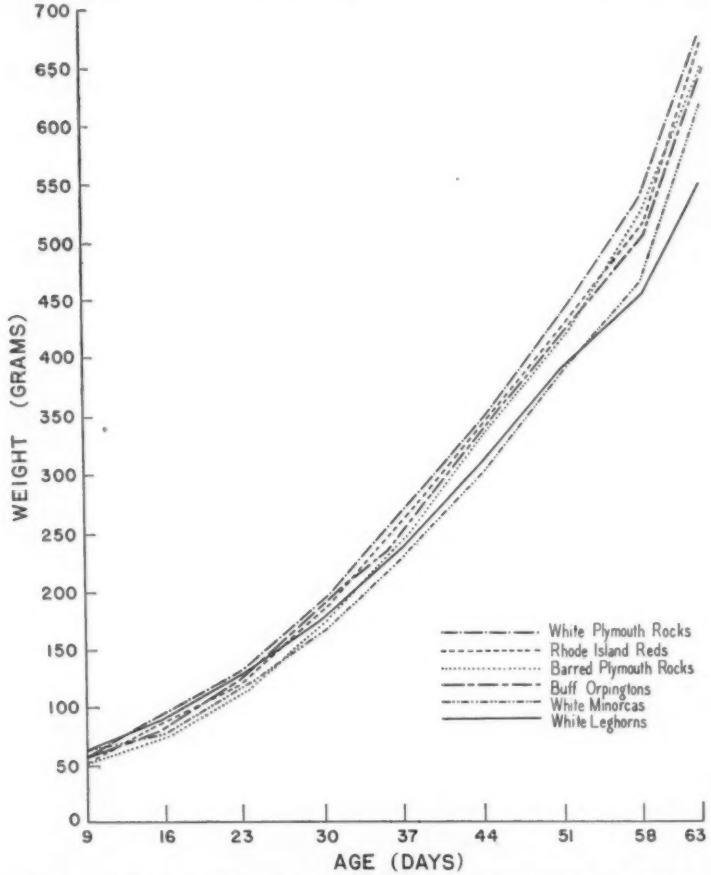

FIGURE 1.—Growth curves of 5 breeds and 2 varieties of young chickens from 9 to 63 days of age.

from the Leghorns averaged 2.80 mm longer, a significant difference (8.12 times the probable error). This comparison again showed that the Leghorns were less resistant to the growth of these nematodes.

The comparison of the worms from the White Minorcas with those from the Leghorns was very similar to the previous comparison; no constant difference occurred in the number of worms, but those from the White Leghorns were 1.68 mm longer. This was a significant difference (table 1).

TABLE 1.—*The resistance to nematodes of White Leghorns as compared with that of 3 other breeds and 2 varieties of a fourth breed of chickens, as determined by the number and length of Ascaridia lineata recovered from each breed at autopsy.*

GROUP A, PARASITIZED AT 12 DAYS OF AGE AND EXAMINED AT 33 DAYS

Breed	Hosts	Worms	Difference	Ratio, $\frac{D}{E}$[1]	Mean length of worms	Difference	Ratio, $\frac{D}{E}$
	Number	*Mean number*			*Mm*		
White Leghorns	46	8.28	3.39	3.03	22.08	4.42	10.83
Rhode Island Reds	35	4.89			17.66		
White Leghorns	46	8.28	1.20	.90	22.08	1.74	3.24
White Plymouth Rocks	36	7.08			20.34		
White Leghorns	46	8.28	.91	.60	22.08	.36	1.12
Buff Orpingtons	36	9.19			22.44		
White Leghorns	46	8.28	.94	.63	22.08	2.80	8.12
Barred Plymouth Rocks	36	9.22			19.28		
White Leghorns	46	8.28	.81	.72	22.08	1.68	4.22
White Minorcas	32	7.47			20.40		

GROUP B, PARASITIZED AT 21 DAYS OF AGE AND EXAMINED AT 40 DAYS

White Leghorns	61	7.34	3.73	4.79	18.19	3.73	8.71
Rhode Island Reds	51	3.61			14.46		
White Leghorns	61	7.34	4.90	7.34	18.19	4.82	5.46
White Plymouth Rocks	55	2.44			13.37		
White Leghorns	61	7.34	3.92	5.38	18.19	.81	1.56
Buff Orpingtons	50	3.42			17.38		
White Leghorns	61	7.34	3.63	5.07	18.19	1.41	2.97
Barred Plymouth Rocks	48	3.71			16.78		
White Leghorns	61	7.34	.66	.72	18.19	2.12	6.63
White Minorcas	40	8.00			20.31		

GROUP C, PARASITIZED AT 30 DAYS OF AGE AND EXAMINED AT 51 DAYS

White Leghorns	49	5.73	2.89	3.79	23.83	3.65	6.78
Rhode Island Reds	43	2.84			20.18		
White Leghorns	49	5.73	2.34	2.99	23.83	3.37	7.61
White Plymouth Rocks	61	3.39			20.46		
White Leghorns	49	5.73	.48	.49	23.83	.09	.21
Buff Orpingtons	53	5.25			23.92		
White Leghorns	49	5.73	3.02	4.14	23.83	4.19	6.22
Barred Plymouth Rocks	52	2.71			19.64		
White Leghorns	49	5.73	3.22	4.53	23.83	4.80	9.72
White Minorcas	49	2.51			19.03		

GROUP D, PARASITIZED AT 33 DAYS OF AGE AND EXAMINED AT 54 DAYS

White Leghorns	51	1.22	0.36	1.18	20.00	3.41	4.61
Rhode Island Reds	59	1.58			16.59		
White Leghorns	51	1.22	.07	1.80	20.00	3.07	3.71
White Plymouth Rocks	61	1.15			16.93		
White Leghorns	51	1.22	.49	1.62	20.00	2.06	2.52
Buff Orpingtons	52	1.71			17.94		
White Leghorns	51	1.22	.05	.02	20.00	2.08	2.57
Barred Plymouth Rocks	51	1.27			17.92		
White Leghorns	51	1.22	.13	.40	20.00	3.74	4.37
White Minorcas	26	1.35			23.74		

GROUP E, PARASITIZED AT 44 DAYS OF AGE AND EXAMINED AT 65 DAYS

White Leghorns	46	2.00	0.64	1.73	21.45	3.87	4.03
Rhode Island Reds	36	1.36			17.58		
White Leghorns	46	2.00	.30	.83	21.45	1.53	2.45
White Plymouth Rocks	37	1.70			19.92		
White Leghorns	46	2.00	1.00	2.99	21.45	5.57	5.57
Buff Orpingtons	21	1.00			15.88		
White Leghorns	46	2.00	1.31	3.99	21.45	4.03	3.91
Barred Plymouth Rocks	35	.69			17.42		
White Leghorns	46	2.00	.81	2.33	21.45	3.74	5.62
White Minorcas	43	1.19			17.71		

[1] The ratio as here used is the quotient of the difference divided by its probable error.

In this experiment the Leghorns were significantly less resistant to the growth of the worms in 4 of the 5 comparisons.

Group B, 40 Days of Age

In the group B series of experiments the chickens were parasitized at 21 days of age and examined when 40 days old.

In both number and length of worms the White Leghorns were constantly less resistant to the parasites than the Rhode Island Reds (table 1).

On comparing *Ascaridia lineata* from the White Rocks with those from the Leghorns an average of 4.90 more was found in the Leghorns, a difference 7.34 times the probable error. The worms from the White Leghorns were also significantly longer than those from the White Rocks, the difference being 5.46 times the probable error. Here again were found constant differences in the number and in the length of worms, which indicated that the White Leghorns were less resistant to *A. lineata* than were the White Plymouth Rocks.

From the Buff Orpingtons there was recovered an average of 3.92 worms less than from the White Leghorns, a significant difference. There was but slight difference between the two breeds in length of worms. This result is similar to that obtained in the comparison of Buff Orpingtons and White Leghorns in group A.

In comparing the Barred Plymouth Rocks with the White Leghorns the latter were found to be constantly less resistant to these intestinal worms (table 1).

The average number of worms in the White Minorcas was approximately the same as that in the White Leghorns, while the average length was greater in the Minorcas than in the Leghorns.

Many of the evidences from this series of experiments point to the White Leghorns as being less resistant to *A. lineata* than were the other breeds and varieties of chickens.

Group C, 51 Days Old

The chickens of the third group were parasitized at the age of 30 days. They were examined when 51 days old.

The White Leghorns again averaged more and longer worms than the Rhode Island Reds. This, together with the results of the previous tests, points to the White Leghorns as being less resistant to these nematodes.

The White Plymouth Rocks showed an average of 2.34 worms less than those in the White Leghorns, a difference which is probably significant (2.99 times the probable error). The White Plymouth Rocks were more resistant also to the growth of *Ascaridia lineata*, the worms from them averaging 3.37 mm less in length than those from the White Leghorns, a significant difference.

As in the two previous groups, no constant differences either in the number or in the length of *A. lineata* were noted between the Buff Orpingtons and the White Leghorns.

Both as to number and length of *Ascaridia lineata*, the White Leghorns were less resistant than were the Barred Plymouth Rocks.

From the White Minorcas 3.22 fewer worms were isolated than from White Leghorns, a difference which was significant; the worms were also shorter by 4.80 mm on an average.

Results in this group gave further evidence that the White Leghorn chickens were less resistant to *A. lineata* than were the other breeds and varieties of chickens.

Group D, 54 Days of Age

The chickens in group D were parasitized at 33 days of age and examined when 54 days old.

Approximately the same average number of *Ascaridia lineata* were isolated from the Rhode Island Reds as from the White Leghorns. However, the worms from the Leghorns averaged 3.41 mm longer, a difference 4.61 times the probable error.

Only slight differences in the average number of worms were found between the White Plymouth Rocks and the White Leghorns, but the worms from the Leghorns averaged 3.07 mm longer than those from the White Plymouth Rocks, a significant difference. In this comparison, as in that between the length of worms from the Rhode Island Reds and from the White Leghorns, the evidence indicates that the White Leghorns were less resistant than either the Reds or the White Rocks to the growth of *Ascaridia lineata* in their digestive tracts.

In the fourth consecutive experiment no constant differences were found between the number and the length of the worms from the Buff Orpingtons and those from the White Leghorns. The *A. lineata* averaged somewhat longer in the Leghorns in this group, but the difference was not significant.

Similar results were obtained in the comparison of the number and the length of the *Ascaridia lineata* from the Barred Plymouth Rocks and those from the White Leghorns. With a larger number of hosts it is probable that the average difference in the length of *A. lineata* in these two breeds would have been significant, as the worms averaged 2.08 mm longer in the White Leghorns, a difference 2.57 times the probable error. Essentially the same average number of *A. lineata* was found in the White Minorcas as in the White Leghorns. The Minorcas were less resistant, as the worms averaged 3.74 mm longer than those from the Leghorns. While there were no essential differences in number of worms isolated from chickens in this group; those from the Leghorns had the greater average length in 4 of the 5 comparisons.

Group E, 65 Days of Age

The fifth experiment was performed on chickens parasitized at 44 days of age and examined when 65 days old.

On counting the worms it was found that the difference between the number of worms isolated from the White Leghorns and from the Rhode Island Reds was negligible, but those from the White Leghorns were 3.87 mm longer on an average than those from the Rhode Island Reds, a significant difference. In this test the White Leghorns had more worms and longer ones, indicating that the Leghorns were less resistant to *Ascaridia lineata* than were the Reds.

Only a slight difference was found between the average number of worms from the White Plymouth Rocks and that from the White Leghorns. The length of the worms was somewhat greater in the Leghorns, and with a larger number of hosts the difference would probably have proved significant.

Slightly fewer nematodes were found in the Buff Orpingtons than in the Leghorns. These heavy Buff Orpingtons proved to be much more resistant than the Leghorns to the growth of the *Ascaridia lineata* as the worms from the Buff Orpingtons averaged 5.57 mm shorter, a significant difference.

The *A. lineata* from the Barred Plymouth Rocks reached the low average of 0.69 worm, an average of 1.31 worms less than that from the Leghorns. This proved to be a significant difference. The nematodes from the Leghorns also averaged 4.03 mm longer than those from the Barred Plymouth Rocks, a significant difference. Here again the indication is that the White Leghorns were less resistant than the heavy Barred Rocks.

The number of *Ascaridia lineata* from the White Minorcas was not significantly different from that from the Leghorns, although the Leghorns had the larger average number of worms. But the worms averaged 3.74 mm longer in the White Leghorns. This difference being significant gives evidence in 3 of the 5 comparisons of length of worms that the White Leghorns were less resistant to the growth of these intestinal worms than were the White Minorcas.

When the average number and length of the *Ascaridia lineata* from the White Leghorns are compared with the number and length of those from the other breeds and varieties of chickens in the five groups of experiments it is found that in 39 of the 50 comparisons the White Leghorns had more or longer worms and that the differences were significant in 25 [3] of the 39 times. Thus, the evidence from these five groups of experiments points unmistakably to the White Leghorns as being less resistant to these intestinal worms both in respect to number and size, than were any of the other breeds or varieties.

Why these fowls should be less resistant is not certain. They mature somewhat earlier than do the other breeds. Likewise, the plumage develops more rapidly in the Leghorns. These characteristics should tend to increase the resistance of the White Leghorns. The only marked difference observable between the White Leghorns and the other breeds was their greater activity in the pens. It is possible that in this greater utilization of nervous energy the White Leghorns correspondingly reduce their resistance to these intestinal parasites.

The fact that in two comparisons the White Minorcas were significantly more susceptible than the Leghorns indicates a possibility of different strains of White Minorcas. The worms from the White Leghorns in group C were somewhat longer than usual, and those from the White Minorcas in group E somewhat shorter, although, of course, the chickens were older and this difference may have been due to age resistance. The worms from the White Minorcas in group D averaged 23.74 mm, which was the largest average worm length from any group of the Minorcas. The differences may have been due to different strains among the *Ascaridia lineata*, but these cultures were made from worms of the same length and frequently consisted of the eggs from one worm. As the cultures were well mixed, one would expect to find longer average worms also in some of the other breeds in group D, but in all cases the average worm length was less in the other breeds or varieties in group D than in the White Minorcas or in the corresponding breeds in group C, which were younger (table 1).

[3] This does not include three comparisons with ratios of 2.97 or 2.99 that were probably significant.

The evidence therefore is indicative of the occurrence of two strains of White Minorcas, one more susceptible than the other.[4]

COMPARATIVE RESISTANCE OF THE OTHER BREEDS AND VARIETIES TO ASCARIDIA LINEATA

Consideration will next be given to the comparative resistance of the 3 breeds and 2 varieties other than White Leghorns as determined by the average number and length of *Ascaridia lineata* obtained from each. The data from the five experiments are given in table 2. As both the number and the length of the nematodes are considered in each group, there are 10 possible comparisons per group for each breed or variety; and in the five groups a total of 50 comparisons.

TABLE 2.—*The comparative resistance to nematodes of 3 breeds and 2 varieties of a fourth breed of chickens as determined by the number and length of the Ascaridia lineata recovered from each breed at autopsy*

Breed	Hosts	Worms	Difference	Ratio, $\frac{D^1}{E}$	Mean length of worms	Difference	Ratio
	Number	*Mean number*			*Mm*		
GROUP A, EXAMINED AT 33 DAYS							
Rhode Island Reds	35	4.89	2.19	2.05	17.66	2.68	4.43
White Plymouth Rocks	36	7.08			20.34		
Rhode Island Reds	35	4.89	4.30	3.28	17.66	4.78	11.25
Buff Orpingtons	36	9.19			22.44		
Rhode Island Reds	35	4.89	4.33	3.41	17.66	1.62	3.66
Barred Plymouth Rocks	36	9.22			19.28		
Rhode Island Reds	35	4.89	2.58	3.17	17.66	2.74	5.65
White Minorcas	32	7.47			20.40		
White Plymouth Rocks	36	7.08	2.11	1.42	20.34	2.10	3.81
Buff Orpingtons	36	9.19			22.44		
White Plymouth Rocks	36	7.08	2.14	1.48	20.34	1.06	1.88
Barred Plymouth Rocks	36	9.22			19.28		
White Plymouth Rocks	36	7.08	.39	.36	20.34	.06	.10
White Minorcas	32	7.47			20.40		
Buff Orpingtons	36	9.19	.03	.02	22.44	3.16	8.66
Barred Plymouth Rocks	36	9.22			19.28		
Buff Orpingtons	36	9.19	1.72	1.30	22.44	2.04	4.92
White Minorcas	32	7.47			20.40		
Barred Plymouth Rocks	36	9.22	1.75	1.38	19.28	1.12	2.58
White Minorcas	32	7.47			20.40		
GROUP B, EXAMINED AT 40 DAYS							
Rhode Island Reds	51	3.61	1.17	1.76	14.46	1.09	1.20
White Plymouth Rocks	55	2.44			13.37		
Rhode Island Reds	51	3.61	.19	.28	14.46	2.92	5.24
Buff Orpingtons	50	3.42			17.38		
Rhode Island Reds	51	3.61	.10	.15	14.46	2.32	4.50
Barred Plymouth Rocks	48	3.71			16.78		
Rhode Island Reds	51	3.61	4.39	4.98	14.46	5.85	13.42
White Minorcas	40	8.00			20.31		
White Plymouth Rocks	55	2.44	.98	1.75	13.37	4.01	4.33
Buff Orpingtons	50	3.42			17.38		
White Plymouth Rocks	55	2.44	1.27	2.34	13.37	3.41	3.78
Barred Plymouth Rocks	48	3.71			16.78		
White Plymouth Rocks	55	2.44	5.56	7.09	13.37	6.94	8.08
White Minorcas	40	8.00			20.31		
Buff Orpingtons	50	3.42	.29	.47	17.38	.60	1.09
Barred Plymouth Rocks	48	3.71			16.78		
Buff Orpingtons	50	3.42	4.58	5.47	17.38	2.93	6.12
White Minorcas	40	8.00			20.31		
Barred Plymouth Rocks	48	3.71	4.29	5.20	16.78	3.53	8.09
White Minorcas	40	8.00			20.31		

[4] The White Minorca chicks all came from the same flock, but those of group D were sired by different and somewhat smaller White Minorca cocks.

TABLE 2.—*The comparative resistance to nematodes of 3 breeds and 2 varieties of a fourth breed of chickens as determined by the number and length of the Ascaridia lineata recovered from each breed at autopsy*—Continued

GROUP C, EXAMINED AT 51 DAYS

Breed	Hosts	Worms	Difference	Ratio, $\frac{D}{E}$	Mean length of worms	Difference	Ratio
	Number	*Mean number*			*Mm.*		
Rhode Island Reds	43	2.84	0.55	0.98	20.18	0.28	0.52
White Plymouth Rocks	61	3.39			20.46		
Rhode Island Reds	43	2.84	2.41	2.95	20.18	3.74	7.06
Buff Orpingtons	53	5.25			23.92		
Rhode Island Reds	43	2.84	.13	.27	20.18	.54	1.17
Barred Plymouth Rocks	52	2.71			19.64		
Rhode Island Reds	43	2.84	.33	.72	20.18	1.15	1.99
White Minorcas	49	2.51			19.03		
White Plymouth Rocks	61	3.39	1.86	2.23	20.46	3.46	7.99
Buff Orpingtons	53	5.25			23.92		
White Plymouth Rocks	61	3.39	.68	1.32	20.46	.82	2.37
Barred Plymouth Rocks	52	2.71			19.64		
White Plymouth Rocks	61	3.39	.88	1.79	20.46	1.43	2.91
White Minorcas	49	2.51			19.03		
Buff Orpingtons	53	5.25	2.54	3.24	23.92	4.28	12.66
Barred Plymouth Rocks	52	2.71			19.64		
Buff Orpingtons	53	5.25	2.74	3.55	23.92	4.89	10.08
White Minorcas	49	2.51			19.03		
Barred Plymouth Rocks	52	2.71	.20	.50	19.64	.61	1.49
White Minorcas	49	2.51			19.03		

GROUP D, EXAMINED AT 54 DAYS

Rhode Island Reds	59	1.58	0.43	1.38	16.59	0.34	0.47
White Plymouth Rocks	61	1.15			16.93		
Rhode Island Reds	59	1.58	.13	.36	16.59	1.35	1.91
Buff Orpingtons	52	1.71			17.94		
Rhode Island Reds	59	1.58	.31	.99	16.59	1.33	1.90
Barred Plymouth Rocks	51	1.27			17.92		
Rhode Island Reds	59	1.58	.23	.60	16.59	7.15	9.52
White Minorcas	26	1.35			23.74		
White Plymouth Rocks	61	1.15	.56	1.80	16.93	1.01	1.26
Buff Orpingtons	52	1.71			17.94		
White Plymouth Rocks	61	1.15	.12	.48	16.93	.99	1.25
Barred Plymouth Rocks	51	1.27			17.92		
White Plymouth Rocks	61	1.15	.20	.60	16.93	6.81	8.12
White Minorcas	26	1.35			23.74		
Buff Orpingtons	52	1.71	.44	1.41	17.94	.02	.03
Barred Plymouth Rocks	51	1.27			17.92		
Buff Orpingtons	52	1.71	.36	.94	17.94	5.80	7.02
White Minorcas	26	1.35			23.74		
Barred Plymouth Rocks	51	1.27	.08	.24	17.92	5.82	7.11
White Minorcas	26	1.35			23.74		

GROUP E, EXAMINED AT 65 DAYS

Rhode Island Reds	36	1.36	0.34	0.83	17.58	2.34	2.52
White Plymouth Rocks	37	1.70			19.92		
Rhode Island Reds	36	1.36	.36	.84	17.58	1.70	1.40
Buff Orpingtons	21	1.00			15.88		
Rhode Island Reds	36	1.36	.67	1.93	17.58	.16	.13
Barred Plymouth Rocks	35	.69			17.42		
Rhode Island Reds	36	1.36	.17	.46	17.58	.13	.14
White Minorcas	43	1.19			17.71		
White Plymouth Rocks	37	1.70	.70	1.91	19.92	4.04	4.14
Buff Orpingtons	21	1.00			15.88		
White Plymouth Rocks	37	1.70	1.01	3.75	19.92	2.50	2.47
Barred Plymouth Rocks	35	.69			17.42		
White Plymouth Rocks	37	1.70	.51	1.75	19.92	2.21	3.57
White Minorcas	43	1.19			17.71		
Buff Orpingtons	21	1.00	.31	.95	15.88	1.54	1.21
Barred Plymouth Rocks	35	.69			17.42		
Buff Orpingtons	21	1.00	.19	.59	15.88	1.83	1.83
White Minorcas	43	1.19			17.71		
Barred Plymouth Rocks	35	.69	.50	2.49	17.42	.29	.28
White Minorcas	43	1.19			17.71		

In reading across table 2, it will be seen that when the Rhode Island Reds are compared with the White Plymouth Rocks, the former have fewer or shorter worms in 7 of the 10 comparisons. Of these 7, only 1 is significant, indicating that no marked difference in the resistance occurred between these two breeds at the different ages.

By comparing the Rhode Island Reds successively through the various groups with the White Plymouth Rocks, Buff Orpingtons, Barred Plymouth Rocks, White Minorcas, and finally with the White Leghorns (table 1), it may be seen that in 34 of the 50 comparisons, the Rhode Island Reds had fewer or shorter *Ascaridia lineata* than did the other breeds with which they were compared. Of these 34 cases, 21 were significant (table 3).

In comparing the average number and length of the *Ascaridia lineata* from the White Plymouth Rocks with those from the Buff Orpingtons (table 2), it is seen that in eight instances fewer or shorter *Ascaridia lineata* were found in the White Plymouth Rocks. Three of these cases represented significant differences. By making similar comparisons of the White Plymouth Rocks with the Barred Plymouth Rocks, White Minorcas, Rhode Island Reds, and with the White Leghorns (table 1) it was found that in 32 of 50 comparisons the White Rocks had fewer or shorter worms. Twelve of these differences were significant, as compared with 21 for the Rhode Island Reds (table 3).

TABLE 3.—*Summary of breed resistance to Ascaridia lineata, in analysis of data from tables 1 and 2*

Breed	Fifty comparisons of the number or length of *A. lineata* for each breed		
	Hosts	Total cases of fewer or shorter *A. lineata* than in the other breeds or varieties	Cases of significantly fewer or shorter *A. lineata*
	Number	*Number*	*Number*
Rhode Island Reds	224	34	21
White Plymouth Rocks	250	32	12
Barred Plymouth Rocks	222	32	13
White Minorcas	190	22	8
Buff Orpingtons	212	19	6
White Leghorns	253	11	2

It was interesting to note that when the average number and length of *Ascaridia lineata* from the Barred Plymouth Rocks were compared with these data for the other breeds, there was exactly the same number of cases, 32, as for the White Plymouth Rocks, in which the number of the worms was smaller or their length shorter than in the other breeds. The number of significant cases, 13, was approximately the same as that for the White Plymouth Rocks (table 3).

Fourth in the list of most resistant breeds were the White Minorcas. When the *Ascaridia lineata* from them were compared with those from the other breeds and varieties, it was found that in 22 of the 50 comparisons, the White Minorcas averaged fewer or smaller nematodes. Of this number 8 cases were considered significant (table 3).

A further examination of tables 1 and 2 shows that the Buff Orpingtons, while being perhaps the heaviest breed studied, were far from being the most resistant to the parasites. Of 50 comparisons with other breeds, only 19 showed fewer or smaller parasites, and of this number only 6 were significant. It was thought that the Buff Orpingtons in group C may have been chilled and that therefore their resistance might have been affected, but another test (group A, table 2) showed that the Buff Orpingtons were quite susceptible to *Ascaridia lineata* both as to number and length of worms.

The standing of the White Leghorns in the list has been discussed in a previous section. In table 1 it is seen that comparatively large numbers of rather long worms from the White Leghorns occur throughout; and table 3 shows that in only 11 of 50 comparisons were the worms from the White Leghorns fewer or shorter than those from the other breeds; furthermore, in only 2 of these cases were the differences significant.

From these tests on 1,351 chickens, each of which was fed 50 ± 5 embryonated eggs of the nematode *Ascaridia lineata*, the authors conclude that the White Leghorns were the least resistant and that the Rhode Island Reds were the most resistant to the worms, the others in order of their degree of resistance being the White Plymouth Rocks and Barred Plymouth Rocks about equally resistant, the White Minorcas, and the Buff Orpingtons. No explanation of these differences is available other than the possibility of the Leghorns' greater utilization of nervous energy resulting in reduced resistance to the worms; or that the White Leghorns being the oldest of these breeds or varieties may have been the normal or more tolerant host of this worm and hence less resistant to it.

AGE RESISTANCE

By arranging according to age the different groups of each breed or variety of chickens and giving the corresponding average numbers and lengths of *Ascaridia lineata*, comparisons are readily made for evidence of age resistance of the chickens to the parasites. It will be noted (table 4) that the breeds in group A, the youngest birds, were examined at 33 days of age and those of group E, the oldest, at 65 days. On examining the average numbers of worms from the White Leghorns it is seen that there was a fairly gradual reduction in numbers of worms from group to group, indicating the development of age resistance as the birds grew older. More or less similar results on numbers of worms occurred for the other breeds or varieties as shown in table 4. Some of these differences are significant. Others, doubtless, would have been with larger numbers of hosts.

The average length of worms of the White leghorns in group A was 22.08 mm and that of those in group B, 18.19 mm, a difference of 3.89 mm, which was significant (table 4). Similar comparisons between the Rhode Island Reds, Buff Orpingtons, White Plymouth Rocks, and Barred Plymouth Rocks in groups A and B are significant. While there are irregularities in the rates of growth, and longer worms in the older groups occur, the evidence points to the development of an age resistance to the growth of these worms. In the Buff Orpingtons the average length of worms decreased 6.56 mm in 1 month, the worms averaging 22.44 mm from chickens 33 days old and 15.88

mm from birds 65 days old. In all cases the worms from the chickens 65 days of age are shorter than those from chickens 33 days old (table 4).

TABLE 4.—*Development of age resistance to Ascaridia lineata in certain breeds and varieties of chickens*

Breed and group	Age when examined	Average of *A. lineata*	Average length of *A. lineata*	Breed and group	Age when examined	Average of *A. lineata*	Average length of *A. lineata*
White Leghorns:	*Days*	*Number*	*Mm*	White Plymouth Rocks:	*Days*	*Number*	*Mm*
A	33	8.28	22.08	A	33	7.08	20.34
B	40	7.34	18.19	B	40	2.44	13.37
C	51	5.73	23.83	C	51	3.39	20.46
D	54	1.22	20.00	D	54	1.15	16.93
E	65	2.00	21.45	E	65	1.70	19.92
Rhode Island Reds:				Barred Plymouth Rocks:			
A	33	4.89	17.66	A	33	9.22	19.28
B	40	3.61	14.46	B	40	3.71	16.78
C	51	2.84	20.18	C	51	2.71	19.64
D	54	1.58	16.59	D	54	1.27	17.92
E	65	1.36	17.58	E	65	.69	17.42
Buff Orpingtons:				White Minorcas:			
A	33	9.19	22.44	A	33	7.47	20.40
B	40	3.42	19.38	B	40	8.00	20.31
C	51	5.25	23.92	C	51	2.51	19.03
D	54	1.71	17.94	D	54	1.35	23.74
E	65	1.00	15.88	E	65	1.19	17.71

Attention should be called to the average length of the *Ascaridia lineata* from the various breeds in group C. Although these chickens are 11 and 18 days older than those in groups B and A respectively, the worms average slightly longer in each case, except in the White Minorcas. Whether this resulted from a more vigorous strain of *A. lineata* or from more susceptible groups of chickens is uncertain. In number of worms, the chickens in group C are relatively high. Thus the Leghorns in group C averaged 5.73 worms, and those in group D, only 3 days older, 1.22 worms. Similarly, the Buff Orpingtons in group C average 5.25 worms, and those in group D 1.71 worms. A similar comparison may be made for the White Plymouth Rocks. A plausible explanation is that in the group-C series of experiments a more vigorous strain of *A. lineata* was utilized (table 4).

DISCUSSION

Experimental evidence is available for showing marked differences in the resistance of different species of animals to the same parasite. The Brahman cattle (*Bos indicus*), as is well known, are able completely to escape parasitism with the cattle tick organism, *Babesia bigemina*, which is very pathogenic to domestic cattle (*Bos Taurus*). Swine are not susceptible to the human ascarid, which is morphologically indistinguishable from that of the pig (*25*). Ackert and Grant (*8*) found that the dog tapeworm *Taenia pisiformis* would grow in kittens but that the specimens were markedly reduced in size in the feline hosts; and Scott (*30*) infected cats with *Ancylostoma caninum* larvae from a dog infestation of this hookworm and found that the worms grew slower and reached a smaller final size in the cats than did similar worms in the dogs.

The present studies, however, including a preliminary report by Ackert, Eisenbrandt, Glading, and Wilmoth (*6*), appear to be the

first to give evidence of different degrees of host resistance between breeds of the same species to helminthic infection.

Breed resistance to bacteria was demonstrated by Lambert (*24*), who infected four breeds of chickens with *Salmonella gallinarum*, producing fowl typhoid. After giving all the same-sized dose, he compared the number of deaths. The lowest mortality occured in the White Plymouth Rocks; next in order were the White Leghorns, White Wyandottes, and the Rhode Island Reds. The only similarity of Lambert's results to those from the present experiments was that the White Plymouth Rocks ranked high among the resistant fowls in each case. In his work the White Leghorns had the second smallest number of deaths, whereas in the present experiments the White Leghorns were the least resistant to *Ascaridia lineata*. His results, however, were based on percentages of deaths, and these on the degree of resistance to parasites.

Other data on the comparative mortality of breeds of chickens are available in the work of Harris and Boughton (*19*), who concluded that the White Wyandottes had a significantly higher death rate during the first laying year than Rhode Island Reds or White Leghorns. These authors inferred that the death rate was somewhat higher in Rhode Island Reds than in White Leghorns.

Dudley (*17*), after a careful study of available data, including that of Harris and Boughton, concluded that there were no essential differences in the death rates of these breeds.

Similar results were obtained by Weaver (*34*), who studied the comparative mortality (due to various causes including parasitism) of White Leghorns and Barred Plymouth Rocks and found no constant differences between the breeds.

Judging from these results, there appear to be no marked differences in mortality in breeds of chickens and little if any relationship between death rates and the degree of resistance of breeds or varieties of chickens to parasitism. In this connection it is interesting to note that while the White Leghorns were the least resistant to the parasites, their death rate in the pens was markedly lower than that of any other breed.

The analysis of the data on the resistance of the White Minorcas to the growth of the *Ascaridia lineata* pointed to the possibility of two strains of White Minorcas. The White Minorcas in groups C and E (table 1) were more resistant than the White Leghorns, but the Minorcas in groups B and D were more susceptible. The Minorcas in groups C and E were secured in 1932–33, whereas those in groups B and D were secured in 1933–34. In both years the chickens came from the same flock; but a change was made in cockerels in the second-year flock. When the growth rates of the White Minorcas in the 2 years were compared, it was found that the White Minorcas used the first year were decidedly heavier than those used the second year, indicating two strains of this breed. They, at least, were of different genetical constitution. Strains of chickens varying in resistance to disease have been found by Roberts (*28*), who secured a 41 percent survival of chickens (infected with *Bacterium pullorum*) from selected stock as compared with a 29 percent survival from random chicks. Lambert (*24*) found slight differences in the degrees of resistance of two strains of White Leghorns to *Salmonella gallinarum* the causative organism of fowl typhoid.

In regard to evidence of age resistance of chickens to *Ascaridia lineata* (table 4) it was found that the worms from the group C chickens were much longer than those from the group B chickens, which were younger. This finding is evidence of a more vigorous strain of *A. lineata* in the group C fowls. Different strains of helminths were detected by Scott (*31*), who found dog hookworms (*Ancylostoma caninum*) that were obtained from a cat infestation to be exceptionally adapted to the cat but almost wholly unable to live in puppies. Scott likewise obtained a dog strain of this parasite that was very viable in puppies but not in kittens.

Explanations of the difference in resistance among the breeds, varieties and strains of chickens to *Ascaridia lineata* are not readily available. For a general review of animal resistance to helminths the reader is referred to Chandler (*16*). A recent review in which immunity to protozoan parasites is stressed has been given by Taliaferro (*32*). Concerning size as a factor, the heavier breeds and varieties: Rhode Island Reds, White Plymouth Rocks, and Barred Plymouth Rocks, were more resistant to the *A. lineata* than the lighter Leghorns and White Minorcas; but the heavy Buff Orpingtons proved to be slightly more susceptible than the White Minorcas. Further evidence of heavier birds being more resistant is afforded by the 1932–33 White Minorcas, which were both heavier and more resistant than were the lighter 1933–34 Minorcas. In general behavior the heavy breeds were comparatively inactive, while the lighter, more susceptible Leghorns and Minorcas were markedly more active and nervous. It is conceivable that this greater utilization of energy might be unfavorable to the development of inhibiting factors.

A possible explanation of the results might be that the chickens tested represented susceptible and resistant strains within the breeds and varieties; but the chickens compared came from more than one flock in all cases except those of the White Minorcas and the White Plymouth Rocks. So, it would appear that these tests were made upon a fair section of fowl population.

It is possible that the theory of the abnormal host postulated by Ransom (*27*) and elaborated by Sandground (*29*) is applicable. On this basis the White Leghorns, which are the most susceptible to the *Ascaridia lineata*, would be considered the normal hosts and the heavy breeds, abnormal or more resistant hosts. The fact that the rather susceptible White Minorcas are a Mediterranean breed and therefore closely related to the White Leghorns lends support to this hypothesis. Furthermore, Ackert and Eisenbrandt (*5*) found White Leghorn chickens to be more susceptible to *A. lineata* than were Bronze turkeys, which likewise might be considered abnormal hosts, especially since they were parasitized with worm eggs from a chicken infestation. But even this does not indicate how the resistance works. As previous studies by Ackert (*1*) and by Porter and Ackert (*26*) have shown that blood loss reduces the resistance of chickens to *A. lineata*, the writers favor the view that resistance is an inhibiting complex which so operates as to retard the growth and development of these intestinal worms. Whether the inhibitors are in the nature of macrophages which may attack the worms when they are in close proximity to the intestinal epithelium, whether the worm devours a host substance that checks its growth, or whether the resistance is due to other factors, is uncertain.

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SUMMARY AND CONCLUSIONS

Evidence is presented for the first time on different degrees of resistance among breeds and varieties of one species to the same helminth. The data supporting the conclusions were derived from experiments on 1,351 chickens of 4 breeds and 2 varieties of a fifth breed given the same numbers of eggs from the nematode *Ascaridia lineata* (Schneider). The criteria for judging the resistance were the average number and length of the *Ascaridia lineata* from each group of chickens under comparison.

Most resistant to the parasites were the heavy breeds and varieties: Rhode Island Reds, White Plymouth Rocks, and Barred Plymouth Rocks; the most susceptible were the White Leghorns, Buff Orpingtons, and White Minorcas. A strain of heavy White Minorcas proved to be more resistant to the *Ascaridia lineata* than a lighter strain of the same breed with different genetic constitution. Factors in the differences in resistance appear to include greater utilization of nervous energy by the most susceptible breed, possible differences in strains within a breed, and the normality or tolerance of the host breeds.

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INHERITANCE OF COLD RESISTANCE IN WINTER WHEAT, WITH PRELIMINARY STUDIES ON THE TECHNIC OF ARTIFICIAL FREEZING TESTS[1]

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INTRODUCTION

When this study of the inheritance of cold resistance in winter wheat was initiated in 1929, it was discovered that few data were available to show the exact procedure which might be followed in conducting artificial freezing tests. While a number of studies had been reported, there were some phases of the technic which had not been fully investigated, and others about which there was much disagreement. Consequently a series of experiments were set up in order to develop methods that would be suitable for the problem at hand. The first part of this paper deals with these experiments in technic, and the second part with the inheritance of cold resistance in winter wheat.

REVIEW OF LITERATURE

The effect of low temperature on plant tissue has been the subject of study for many years. The work of previous investigators has been thoroughly reviewed by many authors, including Harvey (*6*),[3] Newton (*11*), Åkerman (*2*), Martin (*9*), Salmon (*18*); consequently, only a few of the more-pertinent papers will be taken up here.

Hill and Salmon (*8*) and Salmon (*18*) obtained high correlations between the results of artificial freezing of varieties of wheat and the field survival as determined in the uniform winter hardiness nurseries.

Åkerman (*2*, *3*), Maximov (*10*), and Quisenberry (*15*) found that results obtained by direct freezing methods agreed very well with those obtained in field trials.

Martin (*9*) concluded that "freezing under controlled temperatures offers the greatest promise in measuring the hardiness of wheat plants by laboratory methods."

Of recent contributions on the inheritance of winter hardiness, the following are of special interest.

Nilsson-Ehle (*12*) and Åkerman (*1*) crossed two varieties of intermediate hardiness. They obtained some lines that were less hardy and others that were more hardy than the parents, and concluded that winter resistance is a quantitative character, controlled by several Mendelian factors.

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[3] Reference is made by number (italic) to Literature Cited, p. 634.

Gaines, according to Schafer (*19*), reported that winter hardiness reacted as a recessive, since a majority of the F_3 rows showed severe winter injury.

Hayes and Aamodt (*7*), Quisenberry and Clark (*16*), and Quisenberry (*15*), working with different wheat crosses, concluded that winter hardiness is a heritable character and is probably controlled by many genetic factors.

MATERIALS AND METHODS

In all tests dealing with technic, three varieties of wheat having a wide range in winter hardiness were used. These varieties were, in order of ascending hardiness, Poole, Michikof, and Minhardi.

For inheritance studies of cold resistance, crosses between pure lines of Poole and Minhardi were made in 1930. The plants in the F_2 generation were subjected to artificial freezing tests during the winter of 1932–33, and those of the F_3 generation during 1933–34. Because of the unreliability of survival readings when based on single plants, the cold resistance of the individual F_2 plants could not be used as a basis on which to make a satisfactory estimate. Consequently 75 seedlings, grown from the seed of each plant, were subjected to the standard freezing test, and the average estimated survival of the seedlings was used as a basis for determining the cold resistance of each F_2 plant, taken at random. Several F_2 plants that showed wide differences in respect to relative cold resistance, were further tested in the F_3 generation. Each test included the seedlings from 10 F_2 or F_3 plants as well as Poole and Minhardi.

All results herein reported were obtained from wheat seedlings grown, hardened, frozen, and thawed in the greenhouse. The freezing was accomplished in a cold chamber by means of the F_{12} (dichlorodifluoro-methane), direct-expansion refrigeration machine shown in figure 1. The chamber is a well-insulated compartment with inside dimensions of 10 by 4 by 3 feet. The temperature was regulated within a range of $\pm 1°$ F. by a thermometer controller. By means of a recording thermometer the exact temperatures during the course of each test were recorded.

Four-inch clay pots were filled with Crosby silt loam soil, and in each pot 7 seeds were placed to insure a uniform stand of 5 plants per pot. Only wheat seedlings that were grown in the greenhouse during December, January, February, and March were used. The temperature in the greenhouse was maintained at about 60° F., until the seedlings were about 5 weeks old. Eight hours before exposure to the freezing chamber, the soil was saturated with water. At 5 p. m., 180 pots containing the seedlings were placed in the chamber and hardened at $+34°$ for a period of 15 hours. Artificial light from two 200-watt bulbs was used during hardening. At 8 a. m., the next morning, the lights in the chamber were turned out, and the thermometer controller set at $+22°$ for freezing. Within a half hour the temperature in the chamber was 22° and remained until 4 p. m.

Immediately after freezing, the seedlings were transferred to the greenhouse, held at a temperature of about 60° F., and kept well

watered in an attempt to revive them. Seven days after freezing records were taken of the average estimated percentage of survival. The method reported by Quisenberry (*15*) was used in estimating the average survival. This method takes into account not only the number of living and dead plants, but also the degree of injury to surviving plants.

EXPERIMENTS IN TECHNIC

The experiments dealing with technic were conducted over a period of three winters, during which time more than 85,000 seedlings were frozen. The following steps in procedure were studied: (1) Freezing temperatures, (2) hardening conditions, (3) age of wheat seedlings at freezing, (4) moisture content of the soil, and (5) treatment of plants after exposure to freezing. A large number of preliminary

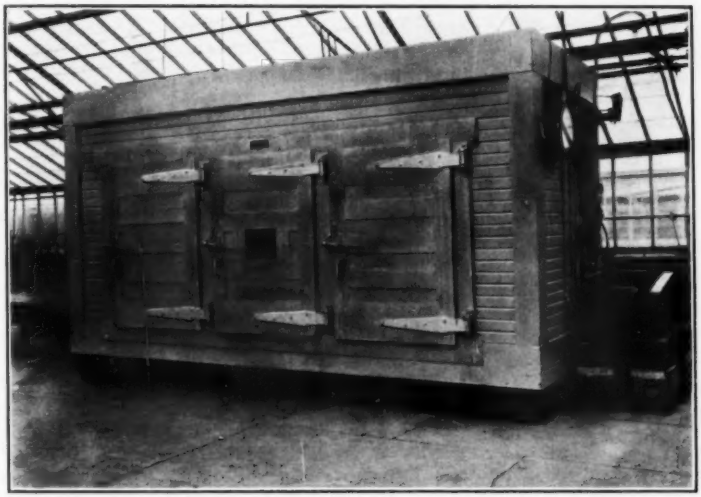

FIGURE 1.—Chamber and compressor used for controlled freezing tests.

experiments were conducted in which various temperatures and duration of exposures were tested in order to obtain information for carrying out the further tests which formed the basis for establishing a standard freezing test. Only the data from these later experiments are reported.

FREEZING TEMPERATURES

From preliminary trials, it was found that the temperature range in which different degrees of injury would result was rather narrow. After this range had been determined a number of tests were conducted to ascertain the freezing temperatures which would express the greatest differential injury between the varieties studied. The data obtained with the three varieties of wheat are shown in table 1.

TABLE 1.—*Influence of freezing temperature on the estimated survival of wheat plants of 3 varieties* [1]

Plants (number)	Freezing conditions		Average estimated survival [2]		
	Temperature	Period	Poole	Michikof	Minhardi
900	°F.	*Hours*	*Percent*	*Percent*	*Percent*
900	18	8	8.1	12.8	22.2
2,700	20	8	5.1	18.3	28.9
900	22	8	21.9	35.3	54.4
900	26	4	27.5	39.0	53.7
	18	4			

[1] Artificial lights were used during hardening, which consisted in exposure to a temperature of 34° F. for 15 hours.

[2] The odds are 19 : 1 for the following differences: 2.95 when comparing 2 means of 900 plants; 2.62 when comparing a mean of 900 with a mean of 1,800; 2.41 when comparing a mean of 900 with a mean of 2,700; 2.27 when comparing 2 means of 1,800; 2.01 when comparing a mean of 1,800 with a mean of 2,700; and 1.71 when comparing 2 means of 2,700.

It will be noted that as the freezing temperatures were increased to 22° F., the estimated average survival also increased. Plants frozen gradually, that is, the first 4 hours at 26° and the second 4 hours at 18°, showed about the same survival as those frozen at 22° for 8 hours. As a result of these tests, a freezing temperature of 22° for a period of 8 hours was adopted as the standard.

HARDENING CONDITIONS

To determine a suitable hardening treatment by which the greatest differential injury could be obtained, wheat plants were subjected to various hardening conditions, as shown in table 2.

An inspection of table 2 shows that wheat plants which were not hardened before being subjected to a freezing treatment of 22° F. for 8 hours showed a much lower survival than those previously hardened at various temperatures for 15 to 39 hours and subjected to the same freezing treatment. Plants hardened at 34° for 15 hours showed the greatest difference in estimated survival between the three varieties representing nonhardy, mid-hardy, and hardy types. Under the conditions of these experiments, it appears that plants hardened for a longer period than 15 hours at a temperature of 34° or higher show a higher estimated survival; however, the differences between nonhardy and hardy varieties are smaller. Therefore a 15-hour exposure at 34° was used as a hardening period. It may be that this period will not prove best under all conditions; for these conditions, however, it serves as a basis for standardizing a suitable technic by which relative cold resistance of wheat seedlings may be determined.

TABLE 2.—*Influence of length of exposure at various temperatures in the hardening chamber on the estimated survival of wheat plants of 3 varieties to artificial freezing at 22° F. for 8 hours*

Plants (number)	Hardening conditions [1]		Average estimated survival [2]		
	Temperature	Period	Poole	Michikof	Minhardi
	°F.	*Hours*	*Percent*	*Percent*	*Percent*
900	([3])	([3])	12.7	26.5	28.1
2,700	34	15	21.9	35.3	54.4
1,800	40	15	40.5	53.3	60.6
1,800	34	24			
1,800	34	39	30.2	38.8	49.6
1,800	36	39	32.5	42.3	51.0

[1] Artificial lights were used during hardening.

[2] See footnote 2 to table 1 for difference necessary for significance.

[3] No hardening; control.

AGE OF WHEAT SEEDLINGS AT FREEZING

To determine the age at which wheat seedlings could be most satisfactorily subjected to comparative cold resistance tests, seedlings of three varieties of wheat ranging in age from 14 to 46 days were grown in the greenhouse and subjected to freezing tests. The estimated average survival of the seedlings is shown in table 3.

TABLE 3.—*Influence of age on the cold resistance of groups of 900 wheat seedlings of different ages and of 3 varieties* [1]

Age of seedlings (days)	Average estimated survival [2]			Age of seedlings (days)	Average estimated survival [2]		
	Poole	Michikof	Minhardi		Poole	Michikof	Minhardi
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
14	0.0	1.0	3.1	32	33.0	32.5	47.7
21	10.3	13.6	20.8	39	23.0	34.6	46.3
24	11.4	22.4	32.4	46	21.0	33.5	50.7

[1] All plants were hardened at 34° F. for 15 hours and frozen at 22° for 8 hours. Artificial lights were used during hardening.

[2] See footnote 2 to table 1 for difference necessary for significance.

The data show that seedlings up to the age of 32 days were quite susceptible to cold. After that period, however, there did not appear to be any difference in cold susceptibility up to 46 days. In making comparative tests for cold resistance, therefore, wheat plants were grown in the greenhouse for about 5 weeks prior to the standard freezing test.

MOISTURE CONTENT OF THE SOIL

There is much disagreement among investigators as to whether plants show a greater injury in dry or wet soil following natural or artificial freezing.

Salmon (*17*) reports a lower survival of barley and oats in dry soil than in medium wet or wet soil during the first season; however, directly contrary results were obtained the second season. Later, Salmon (*18*) found in some tests a greater injury in dry soil than in wet; however, in other tests, where the various lots were frozen slowly, no differences in injury were observed. Sellschop and Salmon (*20*) state that "cowpeas, peanuts, maize, and velvet beans were far more severely injured in wet than in dry soil." Peltier and Tysdal (*14*) and Tysdal (*21*) concluded that plants in dry soil were injured more than those in wet soil.

In experiments conducted by the writer, wheat plants were grown in 4-inch pots, containing Crosby silt loam soil, until 5 weeks of age. Just prior to the freezing test the soil in all of the pots was allowed to dry for about 2 days until it contained 12.4 percent moisture. Eight hours before exposure to the freezing-chamber tests, one-half of the pots were saturated with water and contained 23.5 percent moisture, while the other remained dry. Both lots were then placed in the chamber and subjected to the standard freezing test. The average estimated survival of the three varieties from four tests is shown in table 4.

TABLE 4.—*Effect of moisture content of soil on the estimated survival of groups of 900 plants of 3 varieties of winter wheat by freezing*

Variety	Average estimated survival in [1]—	
	Dry soil	Wet soil
	Percent	*Percent*
Poole	30.5	23.0
Michikof	53.6	37.4
Minhardi	65.0	48.5

[1] See footnote 2 to table 1 for difference necessary for significance.

The data show that, under the conditions of these experiments, winter wheat plants revealed a greater estimated survival in dry than in wet soil. Soil temperatures taken during the course of a freezing test indicated that the temperature fell more rapidly in dry soil than in wet. Since variations in moisture content of the soil produce marked differences in survival, in all comparative hardiness tests all lots were throughly watered 8 hours before a test. Natural drainage left the soil in the different pots at approximately the same moisture content.

TREATMENT OF PLANTS AFTER EXPOSURE TO FREEZING

Anderson and Kiesselbach (*4*), Åkerman (*2*), and Peltier and Tysdal (*13*, *14*) found that slow thawing increased the percentage of survival in plants; Carrick (*5*) and Weimer (*22*) found that slow thawing did not increase the percentage of survival.

To determine the influence of slow and rapid thawing on injury to plants, experiments were conducted in which one-half of the pots, after a regular freezing test, were taken immediately from the chamber to the greenhouse maintained at 60° F., while the other half of the pots were left in the chamber at 34° for 15 hours before they were placed in the greenhouse. The results obtained are shown in table 5.

TABLE 5.—*Influence of the rate of thawing on the estimated survival of groups of 900 wheat plants of 3 varieties* [1]

Variety	Average estimated survival [2]	
	Slow thawing	Rapid thawing
	Percent	*Percent*
Poole	8.9	8.4
Michikof	25.4	26.4
Minhardi	44.5	47.3

[1] Data are not comparable with those in other tables since plants were grown in shaded portion of greenhouse.

[2] See footnote 2 to table 1 for difference necessary for significance.

TABLE 6.—*Estimated survival of parents, and F_1, F_2, and F_3 generations of Poole and Minhardi cross, as determined by controlled freezing tests during 1932–33 and 1933–34*

Parent or cross	Generation	Year	Survival of F_2	Estimated survival for indicated class centers												Lines	Mean	C. V.
				7.5	12.5	17.5	22.5	27.5	32.5	37.5	42.5	47.5	52.5	57.5	62.5			
			Percent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Num-ber*		
Poole	P_1	1933				1	13	8								22	24.1±0.59	11.52
Do	do	1934				2	16	6								24	23.3± .56	11.83
Minhardi	do	1933										5	11	5	1	22	53.0± .85	7.48
Do	do	1934										9	11	4		24	51.5± .72	6.87
F_1	F_1	1934															[1]36.5	
F_2	F_2	1933		1	1	3	10	31	56	82	30	3	2			219	34.6± .44	18.62
3–2	F_3	1934	5.5		8	11	9									28	17.7± .74	22.04
3–1	do	1934	14.6		2	7	16	3								28	21.4± .64	15.73
3–3	do	1934	19.0			1	2	7	6	1						17	28.7±1.14	16.39
3–4	do	1934	27.7				2	5	8	5	2					22	32.5±1.16	16.71
6–55	do	1934	37.7						8	23	6					37	37.2± .50	8.23
3–6	do	1934	40.3							9	19	2	2	1		33	42.5± .80	10.84
7–9	do	1934	48.8								8	23	2			33	46.6± .45	5.58
3–13	do	1934	54.1						2	3	7	11	6	4		33	46.7±1.16	14.21

[1] Average of 34 F_1 plants.

The data in these tests show no significant differences in the estimated survival between slow and rapid thawing of wheat plants. Consequently, in all regular tests, the plants were removed directly from the chamber to the greenhouse.

INHERITANCE OF COLD RESISTANCE IN WINTER WHEAT

In studying the inheritance of cold resistance in soft winter wheat, F_1, F_2, and F_3 generations, together with their parents, were grown in the greenhouse and subjected to artificially controlled freezing tests during the winters of 1932–33 and 1933–34. In this period over 40,600 seedlings were frozen and the cold resistance of each determined. The data obtained are arranged in frequency distributions and shown in table 6.

The results show that the nonhardy parent, Poole, had an estimated survival of 24.1 and 23.3 percent, while the hardy parent, Minhardi, had an estimated percentage survival of 53 and 51.5 for 1932–33 and 1933–34, respectively. Since the coefficients of variability for the parents are small, one might conclude that both parents, though widely different, are quite homozygous for cold resistance. The average estimated survival of 34 F_1 plants tested was 36.5 percent. The estimated survival of the F_2 plants varied from 7.5 to 52.5 percent with an average survival of 34.6 percent; that is, the F_2 plants were about intermediate to the parents in cold resistance. Some lines appeared more susceptible than Poole while others seemed as resistant as Minhardi; but the majority fell into the intermediate classes.

TABLE 7.—*Mean of the estimated survival of several lines in comparison with the survival of the nonhardy or hardy parent*

Parent or cross	Mean survival	Difference between progeny and similar parent	Dif./S. E.
Poole	23.34±0.563		
3–2	17.65± .735	5.69±0.926	6.14
3–1	21.43± .637	1.91± .850	2.25
7–9	46.59± .452	4.87± .852	5.72
3–13	46.74±1.156	4.72±1.363	3.46
Minhardi	51.46± .722		

The data show that the survival found in the F_2 plants agreed very well with that found in the F_3; that is, lines that showed susceptibility to frost injury in the F_2 also were tender in the F_3, while other lines that proved to be cold-resistant in the F_2 were also relatively cold-resistant in the F_3. Some F_3 families appeared to be even tenderer than the nonhardy parent, while others were almost as hardy as Minhardi. The extent to which transgressive segregation appeared and parental types were recovered is shown in table 7.

An examination of these data reveals that strains 3–2 and 3–1, when compared with Poole, show a mean difference of 5.69 and 1.91, respectively. The fact that these differences are 6.14 and 2.25 times the standard error shows that they are significant. This indicates that both strains are less winter-hardy than the nonhardy parent, and strongly suggests transgressive segregation in the direction of

nonwinter hardiness. The two most winter-hardy of the segregates studied (7–9 and 3–13) are less hardy than Minhardi, the hardy parent.

The great increase in the variability between the parents and the F_2 generation, the reappearance of the nonhardy parent, and the recombination of strains less winter-hardy than the nonhardy parent (transgressive segregation) indicate clearly the segregation of genetic factors. The results indicate, therefore, that cold resistance is inherited in the same manner as other quantitative characters; however, the number of genetic factors involved cannot be determined from the data. These results confirm the findings of other investigators, especially those of Nilsson-Ehle (*12*), Hayes and Aamodt (*7*), and Quisenberry (*15*).

SUMMARY

Studies on the technic of control hardiness tests and the inheritance of cold resistance in winter wheat varieties were made on wheat seedlings grown in the greenhouse during the winters of 1931–32, 1932–33, and 1933–34.

In experiments dealing with the development of a suitable technic for measuring cold resistance, over 85,000 wheat seedlings were frozen and their survival determined.

The steps involved in developing a suitable method for determining cold resistance in wheat seedlings under controlled conditions are presented.

Wheat plants hardened for 15 hours at 34° F., under artificial illumination, show wider differences in estimated survival between varieties representing nonhardy, midhardy, and hardy types, than when hardened for a longer period at the same or different temperatures.

Wheat seedlings up to the age of 32 days were much more susceptible to cold than older plants. Wheat plants 32, 39, and 46 days old showed approximately the same survival when subjected to the standard artificial freezing test.

Under the conditions of these experiments, winter wheat plants showed less injury in dry than in wet soil.

The data show no significant differences in the estimated survival between slow and rapid thawing of wheat plants.

In studying the inheritance of cold resistance in winter wheat, over 40,600 F_1, F_3, F_4, and parental plants were subjected to artificial freezing tests, and the estimated survival of each plant determined.

The Poole variety had an estimated percentage survival of 24.1 and 23.3, while Minhardi had an estimated survival of 53 and 51.5 for 1932–33 and 1933–34, respectively. The F_1 plants showed a survival of 36.5 percent.

The estimated survival of the F_2 plants varied from 7.5 to 52.5 percent with an average of 34.6; that is, they were about intermediate to the parents in cold resistance. Families that were less winter-hardy than the nonhardy parent were recombined, which strongly suggests transgressive segregation in the direction of nonwinter hardiness.

The results indicate that cold resistance is inherited in the same manner as other quantitative characters; however, the number of genetic factors involved could not be determined from the data.

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A PHOTOELECTRIC DEVICE FOR THE RAPID MEASUREMENT OF LEAF AREA [1]

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Various methods involving the use of light-sensitive cells as reported by Gerdel and Saulter,[2] Bergman,[3] and others for the determination of leaf area have been described. Most of these methods are based on the use of systems of lenses to secure uniform distribution of the light flux over the aperture upon which the leaves are placed.

The use of lens systems to distribute the light from a single source has two inherent limitations aside from the expense. The amount of energy received by the cell or cells is largely limited by the intrinsic brilliancy of the light source, and therefore necessitates the use of sensitive nonportable galvanometers. The size of the lenses employed also sets a definite limit on the size of aperture which can be uniformly irradiated.

The apparatus to be described in this paper was designed to eliminate the difficulties of optical systems employing large lenses by placing the leaves on a ground-glass diffusing screen and irradiating the diaphragm upon which the screen is set with a highly diffused, uniformly distributed source of radiation.

This method is based on the following theoretical considerations: A group of thin opaque objects such as leaves of a total area A is placed upon and in the same plane as a perfect diffusing screen diaphragmed to a known circular area A_o. The aperture of the diaphragm is irradiated from above by a source of such a flux distribution that the intensity of the flux leaving the ground-glass diffusing screen and reaching the sensitive cells is of the same value at all points of the aperture. When this condition exists, the decrease in response of a linear light-sensitive cell, such as a vacuum photoelectric cell, will be proportional to the opaque areas on the aperture of the ground-glass diffusing screen. The following relationship will then obtain:

$A = LK(I_o - I)$

$A_o = KI_o$ when $I = 0$

Where

A_o = Area of diaphragm of diffusing screen

A = Area of leaves

I_o = Photoelectric current with full aperture of the diaphragm

I = Photoelectric current with leaves in place

K = Constant relating photoelectric cell current and area

[1] Received for publication Jan. 18, 1935; issued June, 1935.

[2] GERDEL, R. W., and SAULTER, R. M., MEASUREMENT OF LEAF AREA USING THE PHOTOELECTRIC CELL. Jour. Amer. Soc. Agron. 20:635-643. ———— 1928.

[3] BERGMAN, H. F., MEASUREMENT OF LEAF AREAS BY MEANS OF THE PHOTOELECTRIC CELL. Report presented at tenth annual meeting Amer. Soc. Plant Phys., Boston, Dec. 30, 1933.

Figure 1 presents in diagrammatic form the details of this instrument, and figure 2 is from a photograph of the apparatus set up for operation.

The illuminator consists of a set of lamp bulbs mounted on an open board above a plate of Florentine or maze glass. The square galvanized-iron housing has a flange at the bottom upon which the glass rests, with a second flange near the top so placed that the lamp bulbs are from 3 to 4 inches above the diffusing glass when the lamp mounting is resting upon it. The lamp mounting consists of 12 receptacles arranged on a board as shown, with a square opening in the center and the corners removed for insuring proper ventilation. The 20 lamp bulbs are mounted in the 12 receptacles by the selection

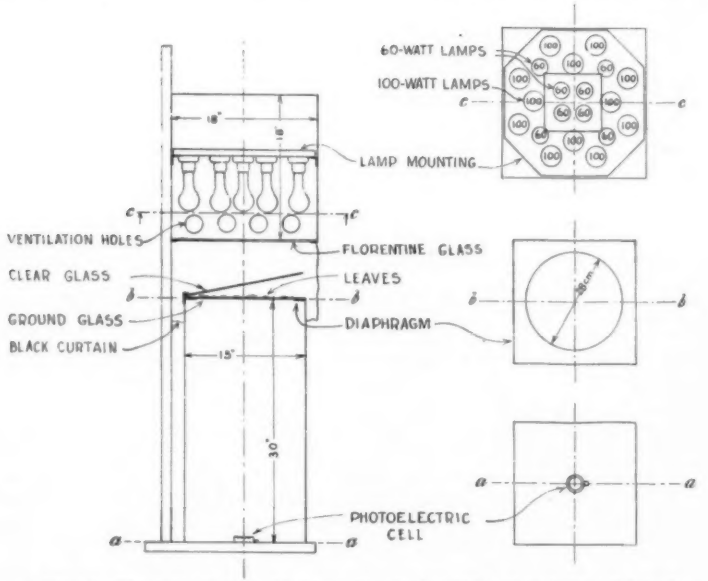

FIGURE 1.—Diagrammatic view of leaf-area apparatus in which a photoelectric cell is used.

of suitable Y and offset double sockets. The use of double sockets is not as satisfactory as small receptacles mounted rigidly on an open metal framework. However, they do lend flexibility to an experimental set-up.

The arrangement is not very critical, the only requirement being that the lamps should be evenly distributed over the area, with the higher wattage lamps around the outside. In this manner, the flux is roughly controlled to give a final distribution which is uniform over the surface of the aperture. The final adjustment is made by raising and lowering the illuminator, which is mounted on a wooden frame above the receiving unit. Ventilation is taken care of by a series of 2-inch holes in the lower part of the housing. This is an important factor and must be given careful consideration, for the apparatus consumes nearly 1,700 watts. The sockets should not

have wax plugs around the screws since the heat melts the wax and it runs down onto the bulbs. The inside of the housing may be painted with a heat-resisting aluminum paint which reflects the radiation and prevents the housing from becoming excessively hot.

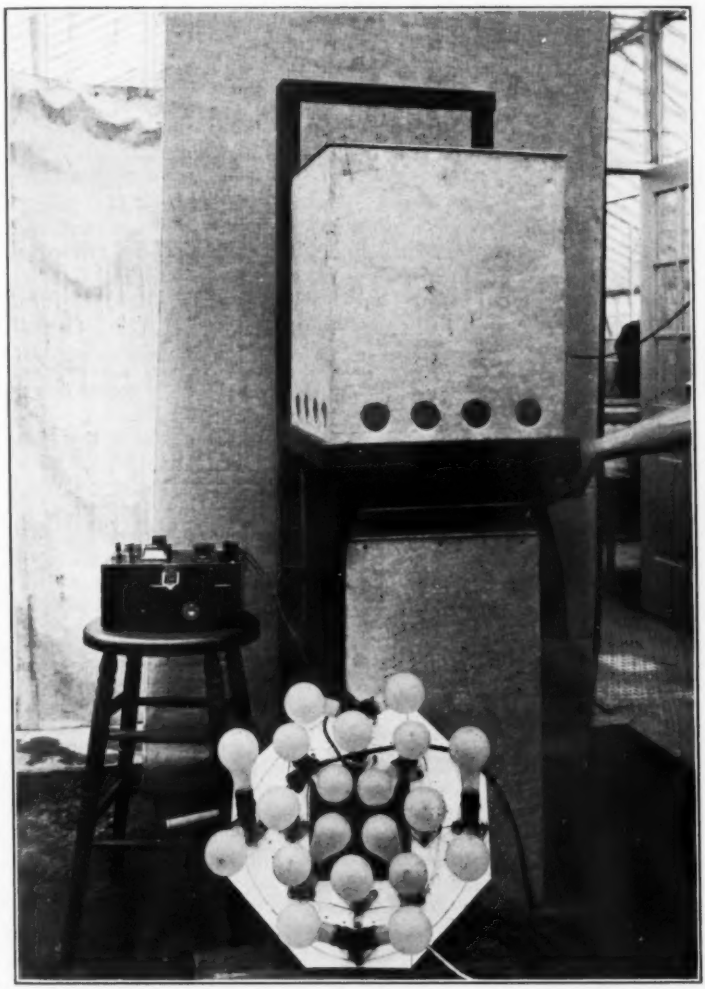

FIGURE 2.—Photograph of leaf-area apparatus set up for operation, with lamp mounting removed from illuminator to show arrangement of lamp bulbs. Potentiometer of portable type on stool.

With one of the earlier instruments, a 1,000-watt lamp in various reflector types was used, but apparently it was not possible to secure the proper distribution of flux with this lamp. A high-wattage lamp would be much more efficient than the lower wattages and much

simpler to use if the proper reflector design could be worked out. Such a reflector would need to give a flux distribution that would be lower in the center than around the periphery of the illuminated area in the plane of the glass-diffusing screen.

The lower receiving unit is smaller in section than the illuminator. The aperture as shown in section *b* of figure 1 is 12 inches in diameter. The diaphragm is covered by a pane of ground glass and a pane of clear glass. The leaves are placed between the two panes and in contact with the ground surface. A flange soldered on the rear of the diaphragm facilitates raising and lowering the clear glass pane for insertion of the leaves.

The light-sensitive unit is mounted in the base. One or more cells may be employed. However, the use of three or more cells increases the over-all accuracy of the instrument, and also makes it possible to install a less sensitive galvanometer. The use of many cells allows a certain degree of adjustment for nonuniform flux distribution, by adjusting the distance of the cells from the aperture and the distance of the cells from one another. It also makes it possible to mount them quite close to the aperture and thus greatly reduce the height of the apparatus.

The determination of flux distribution is made by passing an opaque diaphragm with a small opening over the aperture. It has been found that if the reading on a galvanometer in the cell circuit does not vary more than 10 percent as the opening is moved over the aperture, the resultant accuracy of the entire apparatus will be from 1 to 2 percent of the actual area for areas of from 100 to 500 cm^2 when a single Weston photronic cell and a small portable galvanometer and potentiometer are used. The control of flux distribution is largely made by raising and lowering the illuminator.

The apparatus is calibrated by taking readings with a series of square or circular pieces of opaque material such as thin metal or black bakelite. These pieces are of known areas. With these data and the value of A_o and I_o, a calibration curve can be drawn relating area and photoelectric current.

In practice, the instrument should be calibrated every time the value of A_o has changed appreciably. The calibration requires only a short time and serves to check the changes in line voltage. Under conditions of seriously fluctuating line voltage, it may be advisable to recalibrate with a few known areas with each area measurement. If the line voltage is constant and the apparatus has had 20 to 30 minutes to come to equilibrium, the A_o reading will remain constant for long periods. The use of a voltmeter in the line supplying power to the lamps is not of much value, since a change in voltage sufficient to vary the voltmeter reading very slightly will produce a large change in visible radiation emitted by the lamps. The constancy of the electromotive force generated by the light-sensitive cell is the best indication of steadiness of line voltage.

If the response of the cell is not strictly proportional to the intensity of the light falling upon it, the foregoing relationships do not hold and the calibration curve will not be a straight line having a negative slope, but will be somewhat curved. A pair of typical calibration curves exhibiting the linear and nonlinear conditions is shown in figure 3.

These curves were taken with a potentiometer across a photogalvanic type of cell (Weston photronic). In the first case, the cell is operating with a resistance of 50 ohms shunted across it, thus making the response essentially linear. In the second case, no current is flowing through the cell, and the result is the curved or nonlinear voltage response.

The voltage response of the photogalvanic type of cell is high and the internal resistance is relatively low as compared to the photoelectric cell. This makes it well adapted to potentiometric measurements. However, these cells have two unfortunate characteristics. They have a very nonlinear voltage response, the electromotive force falling off from a straight line with increasing light intensities and the curve approaching an asymptote at high values. If a low resistance of less than 50 ohms be placed across the cell, the response becomes essentially

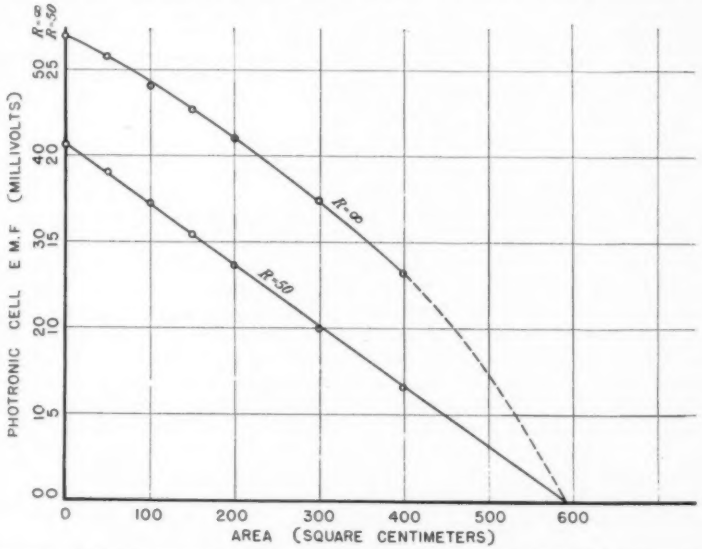

FIGURE 3.—Calibration curves of leaf-area apparatus, showing linear and curved type of responses. *R* is value of resistor across photronic cell.

linear for low light intensities due to the change in internal resistance with light intensity. However, the internal resistance reaches equilibrium rather slowly, and in some cases the last few percentages of change require several minutes to reach their final value. This makes the cell entirely too sluggish for precise work when operating under linear conditions. When a low resistance is used across the cell, the external electromotive force is very greatly reduced, in some cases as much as 90 percent. This adds further to the difficulty of reading. Therefore, it is generally more convenient to use a potentiometer, the reading of which is not influenced by the resistance of the cell.

The output of the photogalvanic type of cell may also be read directly with a sensitive microammeter of fairly low resistance, as is done in the case of some of the direct reading illuminometers which have recently appeared upon the market. With one of these illuminome-

ters or a combination of several photogalvanic cells and a suitable microammeter, the apparatus may be very conveniently calibrated in microamperes or foot-candles.

For the precise determination of area, the use of one or more vacuum photoelectric cells is to be recommended. Under these conditions, the radiation from the Mazda lamps should be more highly and uniformly diffused by the use of two or more layers of diffusing glass spaced an inch or two apart. The modern, well-designed vacuum photoelectric cell, when properly used, is stable and linear in its characteristics. Its main disadvantages lie in the necessity for use of highly sensitive galvanometers or vacuum tube amplifiers.

The leaves of many plants are not, even for practical considerations, entirely opaque and some light passes through them. Thus the areas as read from the calibration chart are incorrect, being lower than the actual value. Therefore, a correction factor must be applied. If the leaves being measured have an average total transmission of T for the light source and cell used, and A' is the observed area, the true area A can be shown to have the following value:

$$A = \frac{A'}{1-T}$$

Thus, the true area is the observed area divided by the absorption of the leaf, the latter often being considerably less than unity.

The light transmitted by most leaves as measured by a photogalvanic type of cell with Mazda lamps for a source varies between 0.05 and 0.15 of the incident light. The transmission may be very conveniently determined by removing the cell from its mounting and placing it in a box of opaque material having a small hole in the lid of such a size that one of the leaves to be studied will readily cover the entire opening. This box is placed on the diaphragm beneath the lamp sources and two readings are taken, one with the leaf in place and one without the leaf. This should be repeated several times and the average of the ratio of each pair of recordings determined. The ratio is the transmission T. For these values, it is essential that the cell be operating under linear conditions.

In order to give an idea of the range of magnitude, the following transmission values of a few typical leaves of greenhouse crops, obtained with a Weston photronic cell, are presented:

Plant:	*Transmission T*	Plant—Continued.	*Transmission T*
Aster	0.07	Rose	0.06
Calendula	.08	Stock	.06
Leaf lettuce	.07	Sunflower	.10
Nasturtium	.10	Sweetpea	.10
Pansy	.06	Tomato	.12

It is, of course, to be expected that these values are influenced to a large degree by growing conditions. Thus, for each group of determinations, a new value needs to be obtained. Since the correction factor seldom alters the reading more than 15 percent, it is not necessary to determine the transmission of more than a few leaves with a high degree of accuracy.

Leaves are generally quite irregular areas with a large ratio of periphery to area. Therefore, it was thought that an edge effect might be present which would make the apparent area of a large number of irregular small surfaces different from that of one large surface of equal area. To test this possibility, a square piece of opaque black paper of 400 cm^2 area was placed on the diaphragm and a reading taken. The paper was then cut into a large number of small irregular pieces to simulate leaves and a reading again taken. The test was repeated several times and in no case did the second reading differ from the first by more than a few percent. This indicates that no appreciable edge effect exists.

Although the apparatus which has just been discussed is suitable only for detached leaves, it would be readily possible to adapt the same principles to the design of a small portable unit for measuring leaves on the plant. Such a device could be made with a pair of 45-degree mirrors so placed that the Mazda lamps and the photoelectric cells would not interfere with the remaining portion of the plant.

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